PERIODICAL
Asamushi, Japan. Marine Biolog. Sta.
Bulletin
(1)
8(1)

東北大学

淺虫臨海實驗所報告

(海洋生物時報 改題)

第八卷 第一号

昭和三十一年三月

THE BULLETIN OF THE MARINE BIOLOGICAL STATION OF ASAMUSHI TÔHOKU UNIVERSITY

Vol. VIII, No. 1

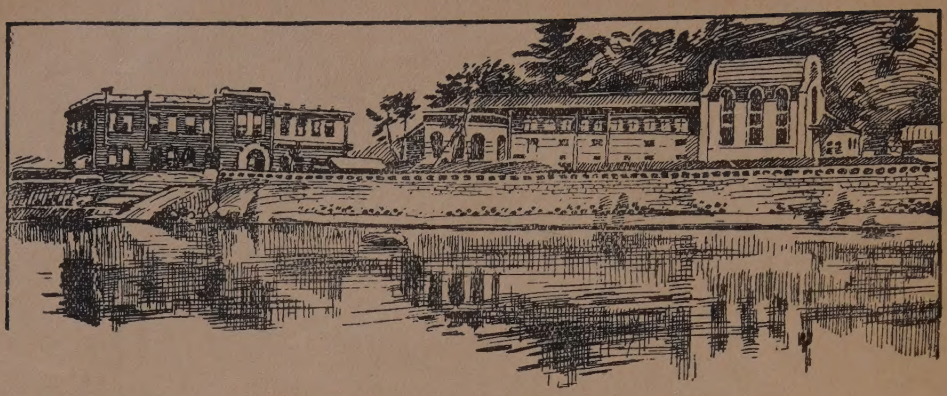
(March, 1956)

SEP 1956
LIBRARY
POFI
FISH & WILDLIFE
SERVICE

Asamushi, Aomori Prefecture
JAPAN

東北大学附属臨海實驗所

青森縣淺虫



ANNOUNCEMENT

The Change of the Japanese Title of the Journal

This journal has been published under the Japanese title "Kaiyô Seibutsu Jihô" ("The Journal of Marine Biology"). This title, however, expresses nothing proper to the Tôhoku University, to which the Station belongs, or to Asamushi where the Station is situated, while the English title "The Bulletin of the Marine Biological Station of Asamushi" seems more appropriate. The Japanese title has, therefore, been changed "Asamushi Rinkai Jikkenjo Hôkoku" in accordance with the English title. The latter will remain unchanged. The series of the new title will succeed the numder of the volume of the previous series.

It may also be announced, by the way, that contributions from the Marine Biological Station have been numbered only when published in the Science Reports of the Tôhoku University (Biology), but those which will be published in this journal will also be numbered hereafter.

本紙改題に就いて

本誌は従来（海洋生物時報）なる誌名を以て刊行されて来たが，此の名称は本実験所の所属する東北大学或は所在地浅虫の何れを示す文字をも含まず従つてその個性を端的に表はさない恨があつた。且つ英文名ともその意義の一致を欠いていた。依つて此度英文名とも一致する表記の題名〔浅虫臨海実験所報告〕と変更した次第である。巻号数は従来の海洋生物時報の巻号数を継承することにした。

因に本実験所業績の番号は従来東北大学理科報告（生物学）に発表の論文にのみ附してあつたが，今後は本誌に発表の論文にも番号を附することにした。

ERRATA

Bulletion of the Marine Biological Station of Asamushi, Tôhoku University. Volume VIII, No. 1, 1956.

Page 34, line 7 from the bottom.
For borh read both

Page 35, line 6 For $MgCI_2$ read $MgCl_2$
For NaCI read NaCl
line 7 For KCI read KCl
For $CaCI_2$ read $CaCl_2$
For $MgCI_2$ read $MgCl_2$
line 10 For 5-70mV read 5-70 mV

Page 38, line 5 from the bottom
For fetween read between

Page 44, Table 1, Water temperature, Feb. 17, 5 M depth
For 5.51 read 5.52
〃 Water temperature, Oct. 1, 5 M depth
For 25.43 read 20.43
〃 Oxygen, Mar. 1, 5 M depth
For 7.17 read 7.19

Appendix : Contents of Volume VII

Page I, No. 1. line 7, Eturô HIRAI
For *Cyathocormus* read *Cyathocormus mirabilis*

Page II, Nos. 2, 3, & 4. line 22
For Head-Frequehcy read Head-Frequency

ERRATA

Bulletin of the Marine Biological Station of Asamushi, Tôhoku University. Volume VII, Nos. 2, 3, & 4. (Memorial Number for the Thirtieth Anniversary) 1955

Page 127, line 5 from the bottom
For Is land read Island

Page 130, line 18
For siland read island

Page 130, line 3 from the bottom
For adta read data

Page 155, Table 1. All the pH values in the table should be corrected by subtracting 0.2.

Page 157, line 7 from the bottom
For iwas read was

Page 171, line 8
For 聯衆 read 聽衆

Page 176

Cover page 4, Contents, line 26, TESHIROGI, W.
For Head-Frequehcy read Head-Frequency

Page 127, Add to the foot-note "Contributions from the Marine Biological Station of Asamushi, No. 214.

BULLETIN OF THE MARINE BIOLOGICAL STATION OF ASAMUSHI,
VOL. VIII, NO. 1. 1956

ON THE SPAWNING OF AN ASCIDIAN, *HALOCYNTHIA RORETZI* (DRASCHE).[1),2)]

By

HIRAI, E. and B. TSUBATA
平井越郎　津幡文隆
Marine Biological Station of Asamushi,
Aomori Prefecture, Japan

Hirai ('41, '51) reported the development and the comparative morphology of the tadpole larvae of a Japanese ascidian, *Halocynthia roretzi* (DRASCHE), Japanese species name Maboya. In his studies, he could get enough eggs to carry out his studies by removing the eggs from the oviduct into the sea water. But by his method it was hard to get so abundant fertilized eggs for the purpose of studies of the development, because the ovarin eggs were not always mature so enough. In foreign ascidians, the spontaneous spawning of Pyuridae and Styelidae (Berrill, '50), *Pelonia corrugata* (Millar, '54) and others has been reported, but that of Japanese ascidians has never been reported. In the early winter of 1955, we could observe the spontaneous spawning of *Halocynthia roretzi* (DRASCHE) and get 100 per cent fertilized eggs at the laboratory of the Marine Biological Station of Asamushi.

Here, we wish to express our thanks to Mr. Shigeo Ôsaka, the master of Nishihama Fishery Cooperative Association and members of the Fishery Administration Section of Aomori Prefectural Government for their kindness of collecting the materials.

MATERIAL AND METHOD

On 11 October 1955, specimens of *Halocynthia roretzi* were dredged from the bottom off Asamushi on the south coast of Mutsu Bay, Aomori Prefecture. This species is cultured for food and attaches to the pine trees which have been placed on the bottom of the sea for the purpose of getting the spontaneous attachments of the tadpole larvae. The specimens of the present observation were two and three years old. Six and four animals three years old were placed in the glass vessel A and B respectively, ten animals two years old in

1) Contributions from the Marine Biological Station of Asamushi, No. 219.

2) Aided by a grant from the Scientific Research Expenditure of the Department of Education.

C, and they were placed under the dropping sea water in the laboratory. During observations no foods were given for them.

OBSERVATION

This species is hermaphrodite as other ascidians, and the sperms as well as the eggs are liberated in the spawning. The spontaneous spawning of the materials has taken place only in three years old animals in the glass vessel A and B, and we could never observe the spawning in the two years old ones in C in this season though they had comparatively ripe eggs. The spawning action took place in almost the same way and in the same period in the vessels of A and B which were placed apart about two metres from each other. As shown in the table the spawning actions were repeated for about an hour from 11.00 a. m. to noon and about eleven to twelve times of actions ot each animal were observed for an hour. Abundant eggs were liberated in the early half of the season in the present study, and were numbered about 12,000 in an hour for an animal, but eggs decreased in number in the latter half of the season.

Table 1.

The spawning period of *Halocynthia roretzi* in the glass vessels A and B.

No. of Observation	Date	Period of Spawning	Water Temp.
1	Nov. 12	Liberated sperm and eggs were found at noon in A.	11.0°C
2	14	11.10 a.m.–noon in A.	10.5
3	16	11.30 a.m.–noon in A.	10.5
4	19	11.15 a.m.–noon in A.	10.0
5	22	11.15 a.m.–noon in A, 10.50 a.m.–11.35 a.m. in B.	10.0
6	24	11.00 a.m.–noon in A and B.	11.5
7	25	A small amount of germ cells were found at 8.00 a.m. in A.	12.0
8	25	11.00 a.m.–noon in A and B.	13.0
9	26	A small amount of germ cells were found at noon in A and B.	11.0
10	Dec. 1	The end of spawning was at 0.40 p.m. in A.	8.6
11	4	11.30 a.m.–0.10 p.m. in A and B.	11.2
12	5	11.10 a.m.–11.50 a.m. in A and B.	12.0
13	6	Liberated germ cells were found at 0.30 p.m. in A and B.	9.7
14	13	11.40 a.m.–0.30p.m. in A and B.	11.0
15	14	A small amount of germ cells were found at 1.00 p.m. in B.	9.5
16	16	Liberated germ cells were found at 0.15 p.m. in A and B.	9.2

The spawning action began at first as a sudden constriction of the branchial siphon, then the body constricted and at the same time the germ cells spurted through the atrial siphon. Soon after the spawning, the constricted

body was restored to the original relieved state (Fig. 1). These about twelve times of spawning action were far more frequent than the usual excretive actions. We also observed germ cells which were liberated along with the slow excretive current of water through the atrial siphon instead of the sudden actions in the period between sudden actions. The eggs were liberated apart from each other by the above mentioned way, but some egg-strings were ex-

Fig. 1. The spontanesous spawning of *Halocynthia roretzi*. about × 1/4.

truded mixed with other free cells. The egg-strings are composed of rows of about two or three eggs which are covered by jelly substance. The length of a string are not constant but they are about 0.7 cm to 3.5 cm in length. The eggs in the string were also fertilized but they survived until only 8 or 16 cell stages.

Hirai ('41) has mentioned in his study on the development that the egg which is removed from the oviduct has a transparent germinal vesicle, and it takes two hours until it matures in the sea water. But in the spontaneous spawning in the present study, eggs soon after being liberated from the atrial siphon have already no germinal vesicle. From this fact, it may be said that the eggs of this species will be liberated after the maturation has occurred in the oviduct in the spontaneous spawning, and also that the eggs in the full growth may mature in the sea water, out of the oviduct. Hirai's ('41) earlier study on the development was carried out with the material from Kesennuma Bay, Miyagi Prefecture, and the breeding season was from December to February. But the breeding season of the present material which was collected from

Mutsu Bay in Aomori Prefecture was November, and it was earlier about a month than in Kesennuma Bay.

The spawning did not occur after 16 of December, but we could observe mature eggs in the gonad though it had decreased in volume. We consider that the spawning will continue longer in animals in the sea bottom than in the animals in the laboratory without foods.

SUMMARY

1. The spontaneous spawning of *Halocynthia roretzi* (DRASCHE) was observed in the laboratory. The sperm and eggs are spurted through the atrial siphon mainly by the jet action caused by the sudden constriction of the body.

2. The spawning action was repeated for an hour from 11.00 a.m. to noon, and the spawning continued from 12 of November to 16 of December, 1955.

3. The breeding season in Mutsu Bay in is about a month ealier than in Kesennuma Bay.

4. The liberated eggs must have already been mature in the gonad, and had no germinal vesicles.

LITERATURE CITED

BERRILL, N.J. 1950 The Tunicata. Ray Society, London.

HIRAI, E. 1941 The early development of *Cynthia roretzi* DRASCHE. Sci. Rep. Tôhoku Imp. Univ., Biology, **16** : 217-232.

———— 1941 An outline of the development of *Cynthia roreizi* DRASCHE. *Ibid.*, **16** : 257-261.

———— 1951 A comparative study on the structures of the tadpoles of ascidians. *Ibid.*, **19** : 79-87.

MILLAR, R.H. 1954 The breeding and development of the ascidian *Pelonaia corrugata* FORBES and GOODSIR. J. Mar. Biol. Ass. U.K. **33** : 681-687.

BULLETIN OF THE MARINE BIOLOGICAL STATION OF ASAMUSHI,
VOL. VIII, NO. 1. 1956

MORPHOLOGICAL OBSERVATIONS OF ASIDIAN LARVAE

2. *CHELYOSOMA SIBOJA* OKA[1),2)]

By

ETURÔ HIRAI
平 井 越 郎
Marine Biological Station of Asamushi,
Aomori Prefecture, Japan

(With one figure)

Chelyosoma siboja OKA, Japanese species name Suboya, is a very abundant solitary ascidian in Mutsu Bay, Aomori Prefecture, and is dredged from the bottom of about 20 meters in depth. In spite of such abundance in Mutsu Bay, the developmental study has never been carried out on this species. The present writer is observing the development of this species, and the present paper deals with the structure of the tadpole larva. The breeding season of this species is from December to January. This species is oviparous and liberates the germ cells spontaneously in the laboratory. The living egg is orange or yellow in colour which differs with individuals. The tadpole larvae hatch out about 45 hours after the spontaneous spawning at 12–13°C, and most of them are metamorphosed within a day at the same temperature.

(1) DIAGNOSTIC CHARACTERS OF THE LARVA

Form and size : External form is of slender type. Total length, *ca.* 1.3 mm, body length, *ca.* 0.4 mm, and 0.15 mm in depth of the body. The living tadpole larva contains orange or yellow yolk granules.

Structures of the larval organs : There are three adhesive papillae which are short and have no stalks at the anterior end of the body. There are eye and static organ in the sensory vesicle, but eye is degenerative and is composed of only 5–6 pigment granules at the postero-dorsal part of the static organ. The epidermal outgrowths are not visible even after the metamorphosis. Tail fin is transparent and is oriented dorsoventral. Notochord is tubular and transparent, but some cellular structures are visible in the posterior part.

Rudiments of the adult organs: The rudiments of the adult organs are

1) Contributions from the Marine Biological Station of Asamushi, No. 220.

2) Aided by a grant from the Scientific Research Expenditure of the Department of Education.

poorly developed. The rudiments of the endodermal organs occupy the most part of the body. A small ectodermal invagination of the rudiment of the branchial aperture is visible in front of the sensory vesicle in the section of the fixed material.

Arrangement of the adhesive papillae: The arrangement is of triangular form, and one side of the triangle is dorso-ventral, the dorsal papilla is on right side, and one corner is observed on the left side which is lower than the dorsal papilla.

Relative position of sensory vesicle, branchial and atrial apertures: It is a type of the linear parallel to the body axis on the dorsal side of the body. One atrial aperture is visible on the dorsal side in the metamorphosing larva.

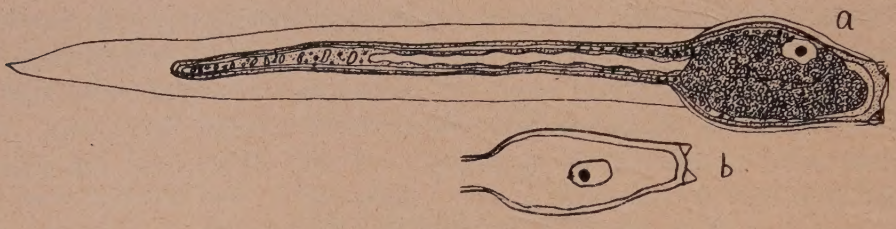


Fig. 1. Living tadpole larva of *Chelyosoma siboja*.
a. right side view, b. dorsal view of body. ×83.

(2) REMARKS

The tadpole larva of *Chelyosoma siboja* OKA resembles that of *Halocynthia roretzi* (DRASCHE) in form and in colour. But the former is a little smaller and has a comparatively stout body than the latter. The degenerative eye with the pigment granules in small numbers is one of the character of this tadpole. On the structures of the larval organs, the rudiments of adult organs, and on the arrangements of organs, the tadpole larva of this species is considered as a typical type of Type I *Cynthia* type, Subtype 1 Simple type (Hirai, '51).

LITERATURE CITED

HIRAI, E. 1951 A comparative study on the structures of the tadpoles of ascidians. Sci. Rep. Tôhoku Univ., (Biol.) **19** (1) : 79-87.

OKA, A. 1935 Report of the biological survery of Mutsu Bay. 28. Ascididae simplices. Sci. Rep. Tôhoku Imp. Univ., (Biol.) **10** (3) : 427-466.

BULLETIN OF THE MARINE BIOLOGICAL STATION OF ASAMUSHI,
VOL. VIII, NO. 1. 1956

MORPHOLOGICAL OBSERVATIONS OF ASCIDIAN LARVAE
3. *CORELLA JAPONICA* HERDMAN, VAR. *ASAMUSI* OKA[1),2)]

By

ETURÔ HIRAI
平 井 越 郎
Marine Biological Station of Asamushi,
Aomori Prefecture, Japan.

(With one figure)

Corella japonica HERDMAN, var. *asamusi* OKA, Japanese species name Asamusiboya, is one of the most common species and the most beautiful of Mutsu Bay ascidians (Oka. A., '35). The materials were dredged from the bottom about 40 meters in depth off Moura in Mutsy Bay on 27 October 1954. The germ cells were removed from the gonads and fertilized eggs were obtained by cross-fertilization. The egg has oval and large follicle cells around the chorion, and is dark yellow in colour. The tadpole larvae hatched out about 20 hours after fertilization at 18°C.

(1) DIAGNOSTIC CHARACTERS OF THE LARVA

Form and Size: External form is of slender type, but the body is a little stout. Total length, *ca.* 0.85 mm, body length, *ca.* 0.22 mm, and *ca.* 0.13 mm in depth of the body. The living tadpole larva is comparatively transparent with dark yellow yolk granules. The postero-dorsal part of the body is especially transparent in the living larva.

Structures of the larval organs: There are three adhesive papillae which are comparatively large but have no long stalks. There are a typical eye and a static organ in the conspicuous sensory vesicle, and the eye is composed of a large mass of pigment granules and lenses. The epidermal outgrowths are not visible. Notochord is tubular and transparent.

Rudiments of the adult organs: The rudiments of the adult organs are poorly developed. The rudiments of the endodermal organs are small and occupy only the central part of the body. The middle part of the endodermal rudiment lacks the yolk granules and is transparent in the living larva.

---

1) Contributions from the Marine Biological Station of Asamushi, No. 221.

2) Aided by a grant from the Scientific Research Expenditure of the Department of Education.

Arrangement of the adhessive papillae: The arrangement is of triangular form, and the two papillae of dorsal side arrange themselves laterally in close contact with each other.

Relative position of sensory vesicle, branchial and atrial apertures: It is a type of the linear parrallel to the body axis on the dorsal side of the body. Two rudiments of atrial apertures are visible on the lateral sides in the metamorphosing larva.

Fig. 1. Left side view of living tadpole larva of *Corella japonica*, var. *asamusi*. ×130

(2) REMARKS

The tadpole larvae of genus *Corella* are found in the figures of *C. willmeriana* (Child, '27) and in *C. parallelogramma* (Knaben, '36). But it is hard to compare the structures of the present species with those of other species, because there are given no details in the morphological descriptions in their studies. The larva of the present species is of Type I *Cyntnia* type, Subtype 1 Simple type (Hirai, '51). The arrangement of the adhesive papillae, the small endodermal rudiments, and the transparent postero-dorsal part would be the main characteristic structures of the larva of this species.

LITERATURE CITED

Child, C. 1927 Developmental modification and elimination of the larval stage in the ascidian, *Corella willmeriana*. J. Morph. **44**: 467–514.

Hirai, E. 1951 A comparative study on the structures of the tadpoles of ascidians. Sci. Rep. Tôhoku Univ., (Biol.) **19** (I): 79–87.

Knaben, N. 1936 Über Entwicklung und Funktion der Testazellen bei *Corella parallelogramma* Mull. Bergens Mus. Arb. Nat. Rek., 5–33.

Oka, A. 1935 Report of the Biological Survey of Mutsu Bay. 28. Ascididae simplices. Sci. Rep. Tôhoku Imp. Univ., (Biol.) **10** (3): 427–466.

BULLETIN OF THE MARINE BIOLOGICAL STATION OF ASAMUSHI,
VOL. VIII, NO. 1. 1956

METABOLIC PATTERNS IN BIVALVES
VIII. DISTRIBUTION OF THE GUANINE-DEAMINATING ACTIVITY IN THE SOFT PARTS OF SOME MARINE BIVALVES

By

SHÛZÔ ISHIDA
石 田 周 三
Biological Laboratory, Chiba University, Chiba, Japan

(With a table and five text-figures)

The author has already reported that the activity to deaminate guanine was found positive in the branchial tissues of the clam, *Meretrix*, but not in those of its foot or mid-gut gland (Ishida 1954 a). He has also investigated the activities of xanthine oxidase (Ishida 1954 c), uricase (Ishida and Tsuzuki 1955), and urease (Ishida 1954 b) in this species. Further he has carried out a comparative investigation on some marine bivalves concerning the urea-deaminating activity in their soft parts (Ishida 1955 b), and has found that mode of distribution of this activity varies considerably from species to species, while in certain species little or no activity of urea-deamination was observed (Ishida 1956).

For a more extensive survey into manners of purine catabolism in bivalves, the author has investigated the distribution of the guanine-deaminating activity in some marine species. The results in the following suggest that guanase is common among these bivalves and is distributed rather universally within their bodies. The localized and exclusive distribution of such activity in the clam gills seems to be exceptional, for in any other species so far investigated this enzyme was found more active in mid-gut gland, and in a less degree in gill and other parts. These results were read bofore the 26th Annual Meeting of the Zoological Society of Japan, Fukuoka, 1955.

Before going further the author wishes to express his cordial gratitude to Professor Shichiroku Nomura for his kind criticisms. He is also grateful to Professor Atsushi Fujita and Mr. Hisayoshi Nagashima of the Chiba University for their generous helpfulness in getting materials. The staff of the Misaki Marine Biological Station of the Tokyo University should be acknowledged. Professor Saburo Isaka should also be acknowledged here, for only with his

help and assistance could the author carry out his work.

MATERIAL AND METHOD

Five Species of marine bivalves were investigated. *Anadara inflata*, *Meretrix meretrix lusoria* and *Venerupis philippinarum* were obtained in the vicinity of the laboratory. The specimens of *Ostrea gigas* were brought in from Goi, Chiba Prefecture and those of *Mytilus edulis* from Misaki, Kanagawa Prefecture.

Like in the case of urea-deamination (Ishida 1955 b), the four organs, *i.e.*, gill, mid-gut gland, mantle, and foot, were investigated. Each of these organs was blotted, weighed (mostly 0.5 to 1.0g), ground in a mortar and was extracted and diluted with $M/5$ boric acid-potassium chloride-sodium carbonate buffer (pH 8.6) to eight or sixteen times the weight in most cases. Then this extract was centrifuged, and the supernatant was used as the enzyme solution.

If specimens were not large enough to give sufficient amounts of tissues from a single individual, two or more individuals were used. This was usually the case with *Venerupis philippinarum* which is relatively small in size.

The substrate solution was made up by dissolving 10 mg of guanine first in four drops of N sodium hydroxide and then in 10 cc of water.

In a small conic flask 0.5 cc of the substrate solution was added to 1 cc of the tissue extract. The final pH reached 9.0. The flask was stoppered loosely and was placed in a bath, of which temperature was kept constant at 38°C. In control flasks the guanine solution was replaced by alkaline water.

After 1 hour of incubation 1 cc of the content was pipetted off into a Conway unit in which ammonia could be driven off with 1 cc of saturated potassium carbonate solution and could be absorbed by diffusion by 1.2 cc of 10^{-3} N sulfuric acid. More highly concentrated acid was used in case ammonia content was higher (Conway 1950).

After standing for 1 hour or so at room temperature the lid of the unit was removed and 1 cc of sulfuric acid which now had absorbed ammonia, was taken from the central well and was diluted, in a test tube, to 5 cc with water. The tube was shaken well to make the content homogeneous before receiving 0.5 cc of Nessler reagent. Using an electrophotometer with a blue filter the per cent transmission of the Nesslerized solution was determined and the ammonia content was calculated and expressed in γ nitrogen per 100 mg wet tissues.

As autolysis may take place in all the tissues and their extracts, the ammonia content in the control flasks may also have increased, more or less, during the period of incubation. This level of ammonia, however, may be exceeded by that in the experimental flasks, if any activity of guanase were exerted. Thus the activity was expressed by the difference between the ammonia

production by the tissues in the presence of guanine and that in its absence.

RESULTS

It will be seen in the table that most of the marine bivalves, so far investigated by the present author, have a common tendency to show the highest activity of guanine-deamination in the mid-gut gland which, however, was quite inactive in the clam, *Meretrix*.

Among the five species investigated, *Anadara inflata* and *Mytilus edulis* belong to Filibranchia, the oyster, *Ostrea gigas*, belongs to Pseudolamellibranchia, and the remaining two, *Meretrix meretrix lusoria* and *Venerupis philippinarum*, belong to Eulamellibranchia. As regards the distribution of guanase, however, little could be discussed in connection with their taxonomic positions, for the clam, for instance, is quite different in this respect from other species including the nearest, *Venerupis philippinarum*.

The results obtained in each of these species will be described in the following. The table (Table 1) and the figures (Figs. 1–5) shall also be referred to.

1. *Anadara inflata* (Fig. 1)

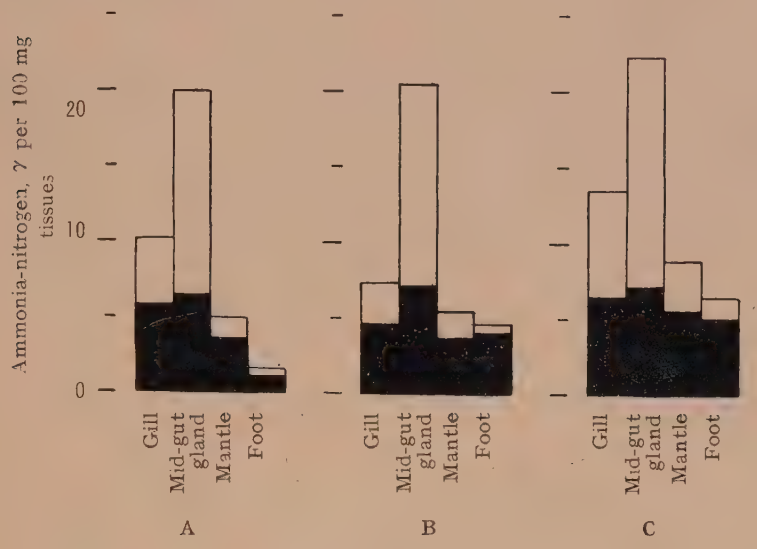


Fig. 1. Deamination of guanine in *Anadara inflata*

In this species the gill lamellae and the mid-gut gland were rather small, and it was often necessary to use two or more individuals to get sufficient amount of tissues of these two organs. In each of the first two cases given in the table two individuals were used. In the third case a single individual was enough to give 1 g each of the organs to be investigated. Each organ was extracted and

diluted with buffer solution to eight times its weight.

The mid-gut gland was found the most active in this animal. The gill was the second and the mantle the third. In the pedal tissues the guanase activity was rather poor in contrast with other three organs.

2. *Mytilus edulis* (Fig. 2)

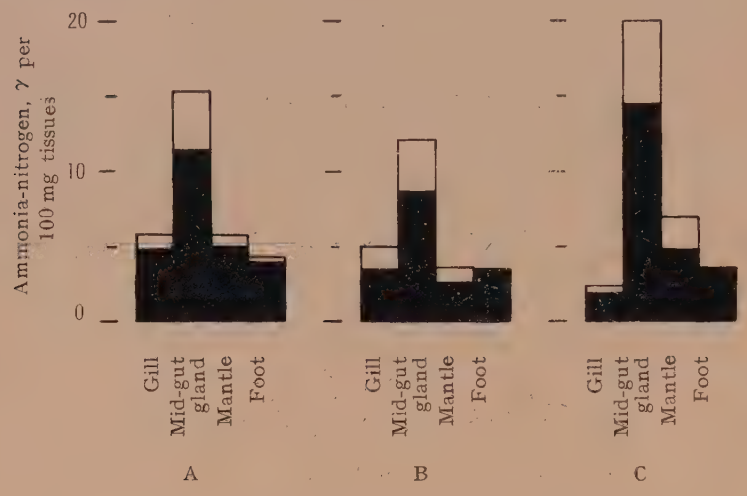


Fig. 2. Deamination of guanine in *Mytilus edulis*

This species also showed the highest activity of guanase in the mid-gut gland tissues, but the extent of deamination was much inferior to that in the preceding species. No distinction could be observed between the gill and the mantle as regards the activity of guanase. The foot showed little or no activity. This last organ is relatively small and loosely structured in this mussel.

In the first two of the three cases given in the table the pedal tissues, which, even if collected from two individuals, could not be obtained amply, were diluteed too much (0.3 g tissues were diluted with 7.7 cc buffer in Case A and 0.2 g with 7.8 cc in Case B), and this caused inaccurate results. Photometric readings for these cases, however, indicated no significant difference between control and experimental preparations, the reciprocal logarithms of per cent transmission being 0.035 *vs.* 0.034 in Case A and 0.026 *vs.* 0.027 in Case B. Only the multiplication, which was necessary to express activities in terms of the unit weight of tissues, has exaggerated these negligible differences. In Case C the pedal tissues weighed 0.5 g and, as the extract was not too much diluted as in the former cases, such an exaggeration could be avoided. In conclusion, not a notable effect of the presence of excess guanine on the production of ammonia was recognized in the pedal tissues of this species.

Table 1

Showing the deamination of guanine in different organs of the marine bivalves.

Species	Case	Ammonia-nitrogen in γ produced by extract of 100 mg tissues of respective organ in 1 hour of incubation at 38°C and pH 9 in the presence (exp) and absence (con) of guanine; and the difference (diff=exp - con)											
		Gill			Mid-gut gland			Mantle			Foot		
		exp	con	diff	exp	con	diff	exp	con	diff	exp	con	diff
Anadara inflata	A[1]	10.2	5.8	4.4	20.0	6.5	13.5	5.0	3.6	1.4	1.6	1.1	0.5
	B[1]	7.4	4.7	2.7	20.5	7.1	13.4	5.5	3.7	2.2	4.6	4.0	0.6
	C[2]	13.5	6.5	7.0	22.3	7.1	15.2	8.8	5.6	3.2	6.5	5.2	1.3
Mytilus edulis	A[3]	5.8	4.8	1.0	15.3	11.5	3.8	5.8	5.0	0.8	4.4	4.0	(0.4)
	B[2]	5.0	3.5	1.5	12.2	8.7	3.5	3.7	2.6	1.1	3.0	3.6	(−0.6)
	C[3]	2.4	1.9	0.5	20.0	14.5	5.5	7.0	4.8	2.2	3.4	3.6	(−0.2)
Ostrea gigas	A[2]	10.1	1.2	8.9	38.4	2.6	35.8	7.7	1.9	5.8			
	B[2]	11.5	2.6	8.9	43.2	2.9	40.3	10.6	0.7	9.9			
Meretrix meretrix lusoria	A[2]	10.0	5.5	4.5	8.2	8.4	(−0.2)	2.2	2.0	(0.2)	1.8	1.9	(−0.1)
	B[2]	9.6	4.8	4.8	5.5	5.5	0	1.3	1.3	0	1.3	1.3	0
	C[2]	16.8	4.1	12.7	13.2	13.2	0	1.9	1.8	(0.1)	1.0	1.0	0
	X[4]			4.6			(−)						0
	Y[4]			10.0									
	Z[4]			6.9									
	W[4]			2.6									
Venerupis philippinarum	A[5]	7.5	7.0	0.5	20.0	14.8	8.4	5.2	4.7	0.5	4.3	3.7	0.6
	B[6]	7.7	7.2	0.5	23.0	13.4	9.6	4.7	4.7	0	3.7	3.7	0
	C[6]	7.4	6.7	0.7	20.0	14.8	5.2	4.9	4.7	(0.2)	4.1	3.7	(0.4)

Note: Figures in parentheses are negligible in respect of technical accuracies.

1) organs from two individuals.
2) organs from a single individual.
3) feet from two individuals, other organs from one.
4) reported already (Ishida 1954 a).
5) mid-gut glands from six individuals, other organs from four.
6) organs from four individuals.

Other three organs were large enough to be investigated more exactly.

3. *Ostrea gigas* (Fig. 3)

According to the results obtained here the oyster seems to have an extraordinarily high activity of guanase in its soft parts, especially in the mid-gut gland. The production of ammonia due to the breakown of guanine by the mid-gut gland tissues in this animal amounted to three to ten times that shown by other active organs in this and other species. Gills and mantles also have shown considerably high activities. The autolytic production of ammonia in this species was rather low in comparison with that in other bivalves (compare the black portions in the figures).

4. *Meretrix meretrix lusoria* (Fig. 4)

That the only site of guanase in this animal was the branchial tissues was the

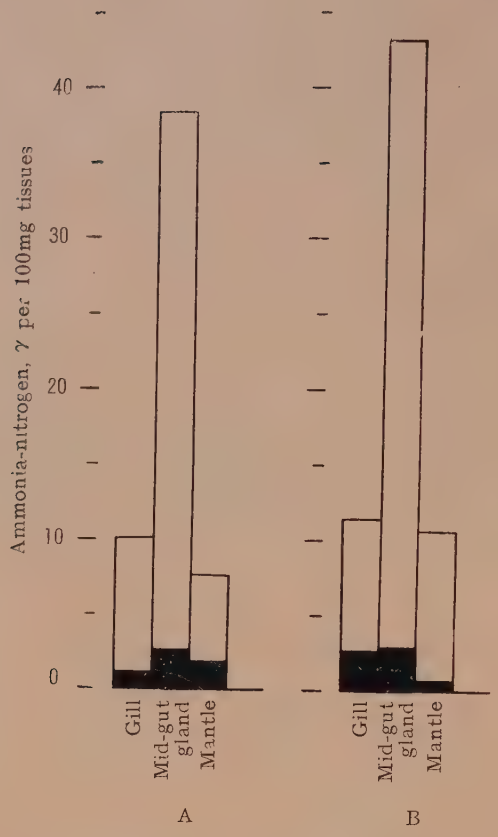


Fig 3. Deamination of guaine in *Ostrea gigas*

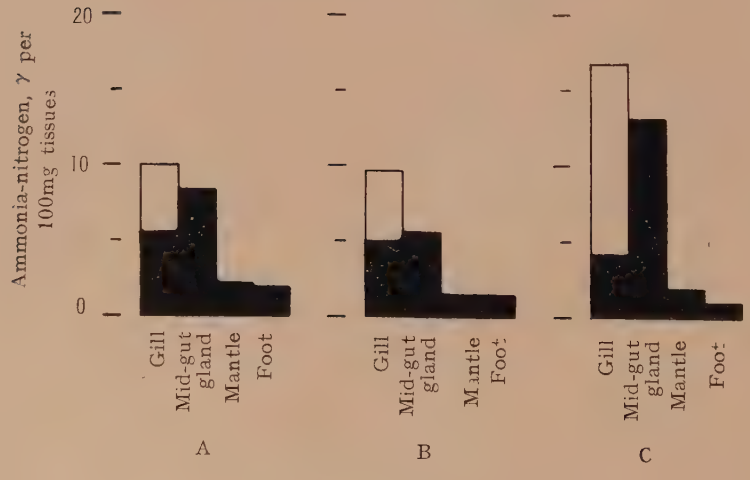


Fig. 4. Deamination of guanine in *Meretrix meretrix lusoria*

main conclusion of the author's first report of this series (Ishida 1954 a). Having investigated certain other species to find out patterns quite different from that of the clam, a few more experiments were felt to be needed to confirm the former results.

As shown in the table, no activity of guanase could be recognized in the mid-gut gland, mantle, and foot. It happened once, in a preliminary test, that the production of ammonia by pedal tissues seemed to have increased in the presence of guanine, but the author has had no opportunity since then to obtain a similar result, and he cannot conclude yet whether this might be an artefact or not. At any rate, this species is very peculiar in showing the highest activity of guanine-deamination in the gill lamellae, and not in the mid-gut gland like in many other bivalves.

5. *Venerupis philippinarum* (Fig. 5)

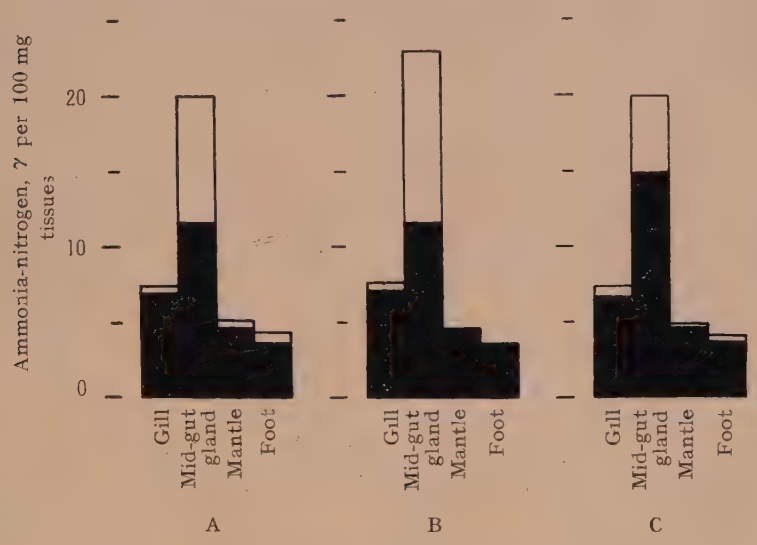


Fig. 5. Deamination of guanine in *Venerupis philippinarum*

Four to six individuals were necessary in this species to collect organs sufficient in amount for determinations. The activity of guanase in this animal is very low in general, and it seems to be rather localized in the mid-gut gland. The data for other organs, especially for foot and mantle, suggest that only very low activities, sometimes nil, could be found in them. As sufficient amounts of pedal and other tissues were available in this species and the extracts being less diluted, even a very small figure in the table is to be noted.

DISCUSSION AND CONCLUSION

In a former work (Ishida 1954 a) the author referred to the conclusion by Florkin (1945) that the presence of adenase and guanase, as well as the lack of nucleoside deaminases, characterizes the invertebrate catabolism of nucleic acids. Then the author put stress upon the importance of the branchial tissues in the clam, *Meretrix*, not only for the respiratory, but also for the excretory functions, for this bivalve showed the activity of deaminating guanine considerably and exclusively in those tissues. The breakdown of urea was also found quite active in the gill (Ishida 1954 b). Urea, however, could also be deaminated in other organs such as foot, mantle, and mid-gut gland ; very remarkably in the last. In another work (Ishida 1955 b) it was shown that the distribution of the urea-deaminating activity varied somewhat from species to species. On the other hand, uricase in *Meretrix* and *Venerupis* was found to be highest in activity in the mid-gut gland and also recognizable in other parts such as gills, mantles and feet (Ishida and Tsuzuki 1955).

All these results of the former works necessarily led the author to think of carrying out some more surveys into distributions of purine enzymes in various species of bivalves, and guanine was chosen as the first to be tested. As mentioned already in the introductory part of this paper, the clam, which has most frequently been employed for experiments by the present author, was found to be rather exceptional in the pattern of the distribution of guanase. It was not in the gill, but in the mid-gut gland that the activity of guanine-deamination was found most active in four bivalved species other than *Meretrix*.

At the very beginning of the present series of works the author repeatedly looked for the activity of guanase in the mid-gut gland of the clam, for this organ looked as if most important above all. He failed. It was rather a surprise that he found the activity exclusively in the gill lamellae of *Meretrix*, and again it is a surprise that he found out the same activity in other organs in related animals.

Having found now the importance of mid-gut gland as well as of gill lamellae, and having found the importance of foot in certain other respects, the author thinks it essential to carry out more investigations on the distribution of enzymes for decomposing purines and their derivatives, for the characteristic patterns for the respective species could be established only through discovering site or sites of catabolism of these substances in various molluscans.

As already cited in a former work the distribution of adenase, guanase, and xanthine oxidase among animal tissues is known to be very erratic (Baldwin 1949). This seems true also within the group of bivalved molluscs, and more descriptions for more species must be necessary to draw, if any, definitive conclusions concerning the occurrence of these enzymes in each of them.

According to the results obtained so far, the mid-gut gland shows a high activity of guanase in many species, and also a remarkable activity of xanthine oxidase and uricase in *Meretrix* (Ishida and Tsuzuki 1955) and other species (Ishida and Tsuzuki 1956). As the site of guanase the gill is important not only in *Meretrix* but also in others. This organ is also important for the deamination of urea in *Meretrix* and other bivalves such as *Anadara*, *Venerupis*, and to a considerable extent in *Mactra* (Ishida 1955 b). Mantle is related more or less with many of the catabolic processes of purines and derivatives. As regards the deamination of guanine, the foot in many bivalves proved to be least active. This organ, however, showed a very remarkable activity of dehydrogenating hypoxanthine in the clam (Ishida 1954 c). Concerning another interesting role of the foot in *Mytilus edulis* descriptions will be given elsewhere (Ishida 1956).

Although the informations at present are quite imperfect, these obtained thus far seem to suggest that purines and their derivatives in molluscan soft bodies might be transferred from one place to another as they follow the course of enzymatical breakdown, perhaps more erratically than in the bodies of vertebrates.

SUMMARY

1. The activity to deaminate guanine in gill, mid-gut gland, mantle and foot in five marine bivalved species was investigated.

2. The mid-gut gland was most active in *Anadara inflata*, *Mytilus edulis*, *Ostrea gigas* and *Venerupis philippinarum*.

3. The gill was active more or less in all of the five species investigated.

4. *Meretrix meretrix lusoria* was exceptional among the five species in showing the activity of guanase only in the gill and not in other organs.

5. The mantle was very active in the oyster, *Ostrea gigas*, considerably active in *Anadara inflata* and *Mytilus edulis*, and slightly active in *Venerupis philippinarum*.

6. Lower activities were shown by feet of *Anadara inflata* and *Venerupis philippinarum*. In *Mytilus edulis* and *Meretrix meretrix lusoria* the feet could be ignored as regards the deamination of guanine.

7. The oyster, *Ostrea gigas*, was most remarkable among the five species in respect of the guanine-deaminating activity.

LITERATURE

Baldwin, E. 1949 Dynamic Aspects of Biochemistry. Cambridge.

Conway, E. J. 1950 Microdiffusion Analysis and Volumetric Error. London.

Florkin, M, 1945 L'évolution du métabolisme des substances azotées chez les animaux. Actualités biochimiques, No. 3. Paris et Liège.

Ishida, S. 1954 a Metabolic patterns in bivalves. I. Distribution of the activity to

deaminate guanine in the soft parts of the clam, *Meretrix meretrix lusoria* (Gmelin). Sci. Rep. Tohoku Univ. 4th Ser. (Biology) **20** : 249–255.

——————— 1954 b Ditto II. Distribution of the activity to deaminate urea in the soft parts of the clam, *Meretrix meretrix lusoria* (Gmelin.). *ibid.* 256–259.

——————— 1954 c Ditto III. Supplementary and miscellaneous notes to the physiology of *Meretrix meretrix lusoria* (Gmelin). J. Coll. Arts and Sci. Chiba Univ. **1** : 41–43.

——————— 1955 b Ditto VI. A preliminary survey into the distribution of the activity to deaminate urea in the soft parts of marine bivalves, *Anadara inflata*, *Mactra sulcataria* and *Venerupis philippinarum*. Bull. Mar. Biol. Sta. Asamushi **7** : 50–56.

——————— 1955 c Ditto VII. Some aspects of the purine metabolism in the clam, *Meretrix meretrix lusoria* (Gmelin). J. Coll. Arts and Sci. Chiba Univ. **1** : 270–271.

——————— 1956 Ditto IX. Some aspects of nitrogen metabolism in marine bivalves, with special reference to the mussel, *Mytilus edulis*. Bull. Mar. Biol. Sta. Asamushi, **8** : 19–32.

ISHIDA, S. and K. TSUZUKI 1955 Ditto V. Distribution of the uricase activity in the bivalves, *Meretrix meretrix lusoria* and *Venerupis philippinarum*. J. Coll. Arts and Sci. Chiba Univ. **1** : 267–269.

——————— 1956 Ditto X. Distribution of the uricase activity in the bivalves, *Mactra sulcataria*, *Anadara inflata*, *Mytilus edulis*, and certain other species J. Coll. Arts and Sci. Chiba Univ. **2** : 79–84.

BULLETIN OF THE MARINE BIOLOGICAL STATION OF ASAMUSHI,
VOL. VIII, NO. 1, 1956

METABOLIC PATTERNS IN BIVALVES
IX. SOME ASPECTS OF NITROGEN METABOLISM IN MARINE BIVALVES, WITH SPECIAL REFERENCE TO THE MUSSEL, *MYTILUS EDULIS*

By

SHÛZÔ ISHIDA
石田周三
Biological Laboratory, Chiba University, Chiba, Japan

(With eight tables)

INTRODUCTION

After finding out the localized distribution of guanine-deaminating activity in the gill lamellae of the clam, *Meretrix meretrix lusoria* (Gmelin), (Ishida 1954 a), the author has carried out several investigations in succession, concerning the catabolism of purines and their derivatives in the soft parts of certain pelecypods. Guanase was found active in the oyster, the marine mussel, and certain clams (Ishida 1956). The activity to dehydrogenate hypoxanthine was found very high in pedal, hepatic, and other tissues of the clam, *Meretrix* (Ishida 1954 c, 1955 c), and somewhat higher in certain tissues of the mussel, *Mytilus* (Ishida and Tsuzuki 1956). This dehydrogenation must be due to the action of xanthine oxidase which will transform xanthine to uric acid.

As is well known, uric acid is the main constituent of excreta in certain animals, especially those which are bound to terrestrial habitats. In such animals this substance with no doubt is synthesized through certain processes, by capturing amino groups derived from amino acids. It is also well known that there is another source from which uric acid is derived. It is from this latter source, the nucleic acid, that man and other primates excrete slight amounts of uric acid. This substance, however, will be decomposed further into allantoin in other mammalian groups, or into simpler compounds in many of the lower animals including certain molluscans.

According to Brunel (1938) the marine mussel, *Mytilus*, seems to be equipped with certain kinds of enzymes which decompose, step after step, purines and derivatives down to simpler substances and finally to carbon dioxide and ammonia. These last substances will be produced by the activity of urease which attacks

urea hydrolytically.

The present author has confirmed that this activity of decomposing urea is remarkable in the gills, mid-gut glands and other parts in the clam, *Meretrix* (Ishida 1954 b). On the other hand, the author and his coworker described a high activity of uricase in the mid-gut gland in the same clam and also in an allied species named *Venerupis philippinarum* (Ishida and Tsuzuki 1955).

On the basis of these findings the author has been believing that all of the marine bivalves will decompose purines to the very last products, carbon dioxide and ammonia, following the ordinary, well-known path from guanine through xanthine, uric acid, allantoin, allantoic acid and urea.

In another work (Ishida 1955 a) the author observed that *Corbicula japonica*, the species inhabiting estuarine, brackish waters, was much more active than *Corbicula leana*, the inhabitant of upper, freshwater streams, in decomposing urea to the simpler products. This result seemed very interesting to the author, for it looked as if to suggest certain relations between the activity of urease in the molluscans and the concentration of salts in their aquatic environment. It was also considered that this result may reinforce, in a certain way, the conclusion of Florkin that the freshwater bivalves, represented in his treatise by *Anodonta*, lack urease which is present in marine species, for instance in *Mytilus* as shown by Brunel (Brunel 1938, Florkin 1945).

Then the author investigated some more bivalves such as *Venerupis philippinarum*, *Anadara inflata*, and *Mactra sulcataria*, all inhabiting sea water. They showed considerable activities of urea-deamination in their gills, mid-gut glands, mantles and feet (Ishida 1955 b).

After having observed these bivalves the author was rather surprised when he found that the urea-deaminating acticity was practically absent from tissues of the mussel, *Mytilus edulis*, the very bivalve which Florkin has chosen as the representative of the marine lamellibranchs (Florkin 1945). First it was considered that certain environmental or physiologic conditions might be related with such an extreme scarcity of urease, but the author has failed to get positive proofs for this. Rather contrarily, most of his successive investigations showed little or no activity of urease in this mussel. Such results will be given presently in this paper.

It was also found that the oyster, *Ostrea gigas*, which was very active in deaminating guanine (Ishida 1956), was devoid of urease, as will also be shown presently.

Thus the author came to the opinion that certain bivalves, like the above two species, might lack urease, or might at least be very short of it, and that they might choose some other way of excreting nitrogen compounds of nucleic acid origin. Further he considers that there might be taking place certain processes

to synthesize uric acid in some bivalves. As will be reported elsewhere (Ishida and Tsuzuki 1956) the activity of uricase seems very slight in the mussel, whereas the xanthine oxidase was found quite active in the same animal. Some of the results shown in the following revealed a considerable amount of uric acid reserved or accumulated in certain parts of the mussel, and suggested the uricotelism in this animal.

This paper deals mainly with the urea-deaminating activity and the distribution of uric acid in some marine bivalves, especially in the mussel, *Mytilus edulis*.

Before giong further the author likes to express his heartiest thanks to Professor Shichiroku Nomura without whose encouragement this paper could not have been completed. Professors Saburo Isaka and Atsushi Fujita in the Chiba University have been very generous to help the author in many ways. They should be acknowledged here. Thanks are also due to Messrs Kiyoshi Tsuzuki and Hisayoshi Nagashima for their assistance.

PART 1. DEAMINATION OF UREA

As cited above, the urea-deaminating activity was very remarkable in some marine bivalves. The species investigated hitherto are *Meretrix meretrix lusoria* (Ishida 1954 b), *Venerupis philippinarum*, *Mactra sulcataria*, and *Anadara inflata* (Ishida 1955 b). It was also found that the brackish species, *Corbicula japonica*, and the freshwater inhabitant, *C. leana*, have the activity of urease (Ishida 1955 a).

In contrast with these the results of determinations in the mussel and oyster were negative in almost all cases, as will be shown in the following.

MATERIAL AND METHOD

The mussel, *Mytilus edulis*, was collected in the vicinity of the Misaki Marine Biological Station, Kanagawa Prefecture. Some of the specimens were kept for days or weeks in the laboratory or in an outdoor pool before use, while some were investigated immediately after being brought into the laboratory.

The oyster, *Ostrea gigas*, was obtained from a bed in Goi, Chiba Prefecture. The determinations were carried out with fresh specimens.

The total soft body, gill lamellae, mid-gut gland, mantle and foot were investigated, whilst the adult oyster lacks the last-named organ.

Each of these organs was weighed, ground in a mortar and extracted with neutral phosphate buffer, of which the volume was always fifteen times that of the tissues. In the case of total body, however, more highly concentrated extracts were used, as will be shown in each place.

The extract was centrifuged and 0.8 cc of the supernatant was placed in a Conway unit in which it was mixed with 0.2 cc of 0.1 per cent urea solution.

This substrate was replaced by water in the control preparation. After 15 minutes of standing at room temperature 1 cc of saturated potassium carbonate solution was added to the above mixture. This reagent will drive off ammonia which will be absorbed by 1.2 cc of $N/1000$ sulfuric acid in the central part of the unit. When the absorption of ammonia was completed about 1 hour after the addition of potassium carbonate, exactly 1 cc of the sulfuric acid was pipetted off into a test tube in which it was diluted with the addition of 4 cc of water. The tube was shaken well and 0.5 cc of Nessler reagent was added. The ammonia content was determined electrophotometrically, and was expressed as nitrogen in γ as produced by extract of 100 mg tissues. The procedure described above is exactly the same as that employed in former investigations in which the same concentration of tissue extract, the same amounts and concentrations of reagents, and the same length of time of reaction (15 minutes) gave the results remarkable enough to show high activities of urease in other lamellibranchs.

RESULTS

Experiment 1 (*Mytilus edulis*)

The specimens used in this experiment were collected on November 11, 1954, and were brought to the laboratory where they were placed in an outdoor pool of sea

Table 1.

Showing ammonia-nitrogen (N) in γ produced by extract of 100 mg tissues of gill, mid-gut gland, mantle, and foot of *Mytilus edulis*, immediately after dissection (0 min.) and 15 minutes (or 30 minutes) thereafter in the presence (exp) and absence (con) of excess urea. Photometric readings (-log T) are also shown. The specimens were kept in normal sea water in the pool for 8 days after collection, then in diluted sea water (Cl=4.9g/L) for 5 days. (Experiment 1).

Organ	Case	Con.						Exp.				
		0 min.		15 mins.		30 mins.		15 mins.		30 mins.		
		−log T	N	−log T	N	−log T	N	−log T	N	−log T	N	Difference exp - con
Gill	A	.014	2.2	.015	2.4			.015	2.4			0
	B	.005	0.8	.005	0.8			.005	0.8			0
	C	.023	3.6			.030	4.6			.025	4.0	(−0.6)
Mid-gut gland	A	.070	10.8	.080	12.4			.080	12.4			0
	B	.050	7.8	.056	8.6			.065	10.2			1.6
	C	.065	10.2			.070	10.8			.069	10.8	0
Mantle	A	.023	3.6	.022	3.4			.019	3.0			(−0.4)
	B	.006	1.0	.005	0.8			.007	1.0			(0.2)
	C	.021	3.2			.030	4.6			.030	4.6	0
Foot	A	.016	2.6	.026	4.0			.015	2.4			−1.6
	B	.013	2.0	.005	0.8			.012	1.8			1.0
	C	.025	4.0			.020	3.2			.023	3.6	(0.4)

Note : The figures in parentheses are within the range of inevitable errors.

water. A heavy rainfall diluted this water so much that the chlorine content decreased down to 4.9 g per liter. This happened on November 19. On November 23, *i.e.*, four days after the dilution of sea water, the specimens were dissected and gills, mid-gut glands, mantles and feet were investigated as regards their activity of urea-deamination. As is shown in Table 1 the activity in these organs appeared to be very low, being nil in most cases.

Except for mid-gut gland the production of ammonia by tissues, either in the presence or the absence of the substrate, was very low, so that the obtained values were less accurate especially when the photometric readings (–log T) were less than 0. 010.

Experiment 2. (*Mytilus edulis*)

As the activity of urea-deamination was not confirmed in the former experiment, and as this negative result might be due to the decreased salinity of the environmental water, some specimens were transferred from the pool into a jar in which sea water with a higher concentration (*ca.* 16 g Cl per liter) was contained, and after 20 or more days, these mussels were investigated. Some of the results in Table 2 may suggest that there seems to have occurred some slight deamination of urea in certain cases, but no positive proofs for this could be

Table 2.

Showing ammonia-nitrogen in γ produced in 15 minutes at room temperature by neutral extract of 100 mg tissues of gill, mid-gut gland, mantle and foot of *Mytilus edulis* in the presence (exp) and absence (con)of urea. Photometric readings (–log T) are also given. The specimens were kept in normal sea water in the pool for 8 days after collection, 5 days in diluted sea water (Cl=4.9 g/L) and then transferred into normal sea water in the laboratory and kept there for 20 days (A) or 21 days (B) or 27 days (C). (Experiment 2)

Organ	Case	Con.		Exp.		Difference, exp - con
		–log T	N	–log T	N	
Gill	A	.035	2.4	.040	3.2	0.8
	B	.059	7.6	.060	7.8	(0.2)
	C	.057	8.8	.056	8.8	0
Mid-gut gland	A	.111	14.2	.125	16.2	2.2
	B	.103	14.4	.108	15.2	0.8
	C	.125	19.4	.125	19.4	0
Mantle	A	.044	3.8	.050	4.8	1.0
	B	.074	10.0	.072	9.6	(–0.4)
	C	.049	7.6	.047	7.4	(–0.2)
Foot	A	.026	1.0	.038	3.0	2.0
	B	.066	8.6	.070	9.2	0.6
	C	.038	6.0	.037	5.8	(–0.2)

Note: The figures in parentheses are within the range of inevitable errors.

given in majority of them.

Experiment 3. (*Mytilus edulis*)

The determination experiments were repeated with the specimens of *Mytilus edulis* which were kept moist under the covering of sea weeds during the hours of transportation from seaside to the laboratory. Either in June (Cases A and B) or in October (Cases C and D) no effect of the presence of excess urea was observed (Table 3.)

Table 3.

Showing ammonia-nitrogen in γ produced in 15 minutes at room temperature by neutral extract of 100 mg tissues of gill, mid-gut gland, mantle, foot and total soft body of *Mytilus edulis* in the presence (exp) and absence (con) of urea. Photometric readings (−log T) are also given. The specimens were investigated immediately after transported to the laboratory under the moist covering of sea weeds. Determinations for Cases A and B were carried out in early June, for C and D in October. (Experiment 3)

Organ	Case	Con		Exp		Difference, exp - con
		−log T	N	−log T	N	
Gill	A	.010	1.6	.013	2.0	(0.4)
	B	.010	1.6	.010	1.6	0
	C	.035	2.2	034	2.2	0
Mid-gut gland	A	.050	7.8	.050	7.8	0
	B	.050	7.8	.051	8.0	(0.2)
	C	.160	22.6	.159	22.4	(−0.2)
Mantle	C	.053	5.2	.053	5.2	0
Foot	C	.025	1.5	.025	1.5	0
Total soft body	D	.132	2.3	.132	2.3	0

Note: The figures in parentheses are within the range of inevitable errors. Due to instrumental disorders the activity of mantle and foot in Cases A and B could not be determined. Total soft body (7.3 g) was extracted with 7.3 cc of buffer solution.

It is very hard to prove absence of a certain enzyme in a certain animal, but it may be said at least that the mussel showed hardly any sign of urea-deaminating activity under the conditions which allowed other kinds of bivalves exert that activity very remarkably.

Experiment 4. (*Ostrea gigas*)

As shown in Table 4 the oyster, *Ostrea gigas*, did not show any sign of the activity of urea-deamination in any part of the soft body.

Table 4.

Showing ammonia-nitrogen in γ produced in 15 minutes at room temperature by neutral extract of 100 mg tissues of gill, mid-gut gland, mantle and total soft body of *Ostrea gigas* in the presence (exp) and absence (con) of urea. Photometric readings (−log T) are also given. (Experiment 4)

Organ	Case	Con		Exp		Difference, exp - con
		−log T	N	−log T	N	
Gill	A	.036	2.6	.037	2.8	(0.2)
	B	.040	3.2	.040	3.2	0
Mid-gut gland	A	.054	5.4	.052	5.2	(−0.2)
	B	.060	6.4	.062	6.8	(0.4)
Mantle	A	.036	2.4	.035	2.2	(−0.2)
	B	.044	3.8	.042	3.4	(−0.4)
Total soft body	C	.161	4.3	.159	4.2	(−0.1)
	D	.136	3.5	.136	3.5	0

Note: The figures in parentheses are within the range of inevitable errors. Total soft body was extracted with twice amount of buffer (2.5 g in 5.0 cc buffer in case C and 4.3 g in 8.6 cc in Case D).

PART 2. CONTENT OF URIC ACID IN VARIOUS PARTS IN THE MUSSEL AND CERTAIN OTHER BIVALVES

The absence of urea-deamination in the tissue extract of the mussel and the oyster suggested that certain kinds of bivalves might be quite different from others in their mode of nitrogen metabolism, or they might adopt more than two ways of catabolising nitrogen compounds. As will be shown elsewhere (Ishida and Tsuzuki 1956) the mussel and the oyster are less active than the clam and other eulamellibranchs in decomposing uric acid. It was also observed, by chance, that the uric acid content of the mid-gut gland extract of the mussel increased, though slight in amount, during the night of standing. It needs many more experiments to confirm the possible synthesis of uric acid in this mussel, but the above incidental observation led the author and his coworkers to the opinion that some attempts should be made to find out the fate of uric acid in the physiology of this animal. First, one of them scraped the inside surface of the shell valves and collected a little of white powder on which Folin's uric acid reagent was applied. The reaction was positive.

Then the author carried out a few preliminary tests and found that the byssus also gives a remarkable coloration in reaction to Folin's reagent. He observed, however, that the shell valves of other lamellibranchs are also positive in this reaction, though seemingly in a less degree than those of the mussel are.

In order to establish the characteristic occurrence of uric acid in the mussel the following experiments were carried out.

MATERIAL AND METHOD

Shell valves of *Mytilus edulis*, *Ostrea gigas*, *Anadara inflata*, *Mactra sulcataria*, *Meretrix meretrix lusoria*, and *Venerupis philippinarum* were investigated. Soft parts of *Mytilus edulis* and *Ostrea gigas*, as well as byssal tufts of the former species were also investigated. Specimens of *Mytilus edulis* were collected in the vicinity of Misaki Marine Biological Station and at Kurihama, Kanagawa Prefecture, those of *Ostrea gigas* were obtained from Goi, Chiba Prefecture, while all the other species were obtainable in Inage, Chiba Prefecture.

The shell valves were crashed and pulverized in a mortar and were extracted with 3.5*N* sulfuric acid for a few hours on a boiling water bath. The extract was filtered and was denatured with 20 per cent trichloroacetic acid solution. The byssal tufts and soft parts were treated in a similar way without being ground. After standing overnight the solution was filtered and 1cc of the supernatant was pipetted off into a 25 cc measuring flask in which it was mixed with 10 cc of urea-sodium cyanide solution and 2 cc of Folin's uric acid reagent. About 60 or more minutes after the addition of water up to the 25 cc mark the blue color was determined photometrically. The uric acid content was expressed in γ per 100 mg material.

RESULTS

Experiment 5.

Anadara inflata, *Mytilus edulis*, *Ostrea gigas*, *Mactra sulcataria*, *Meretrix meretrix lusoria* and *Venerupis philippinarum* were investigated.

In a conic flask 0.5 g of pulverized shell valve or byssal tuft was extracted

Table 5.

Uric acid content, γ per 100 mg, in pulverized shell valves of marine bivalves and in byssal tuft from *Mytilus edulis*. For details of experimental procedure see the text. (Experiment 5)

Species	Organ	Uric acid γ per 100 mg
Anadara inflata	shell valve	10.2
Mytilus edulis	〃	20.4
	〃	15.3
	byssal tuft	178.5
Ostrea gigas	shell valve	6.1
Mactra sulcataria	〃	7.7
Meretrix meretrix lusoria	〃	10.2
Venerupis philippinarum	〃	10.2
	〃	7.7

with 8 cc of 3.5 *N* sulfuric acid, for 1 hour on a boiling water bath. The extract was centrifuged. To 3 cc supernatant 1.5 cc of 20 per cent trichloroacetic acid solution was added. After standing overnight the solution was filtered off and the uric acid content was determined.

It is evident in Table 5 that mussel, *Mytilus edulis*, is exceedingly rich in uric acid. Very remarkable is that the byssal tuft contains uric acid about ten times that found in shell valves.

Experiment 6.

The mussel and the oyster were compared as regards the uric acid content in shell valves, byssal tufts and soft bodies. To 1 part of soft body 9 parts, to 1 part of shell valve 19 parts, and to 1 part of byssal tuft 199 parts respectively of 3.5 *N* sulfuric acid were applied. They were heated for 2 hours on a boiling water bath. The solution was filtered off and was denatured with the equal amount of 20 per cent trichloroacetic acid solution.

Table 6 shows the results.

Either in shell valves or in soft parts the mussel contains a definitely larger amount of uric acid than the oyster. The byssal tuft of the mussel showed an exceedingly high content of uric acid as compared with shell valves.

Table 6.

Uric acid content, γ per 100 mg, of shell valves and soft bodies of *Mytilus edulis* and *Ostrea gigas*. For details of experimental procedure see the text. (Experiment 6)

Species	Organ	Uric acid γ per 100 mg
Mytilus edulis	shell valve	32.0
	〃	30.0
	〃	38.8
	soft body	20.4
	〃	22.0
	〃	22.4
	byssal tuft	90.0
Ostrea gigas	shell valve	10.8
	〃	9.6
	〃	12.0
	soft body	17 8
	〃	13.2
	〃	13.2

Experiment 7.

In order to look into the distribution of uric acid among various parts of the mussel, gill lamellae, mid-gut gland tissues, foot, total soft body of which

byssal tuft was removed, byssal tuft, and shell valves were investigated. To 1 part of byssal tuft 49 parts of sulfuric acid (3.5 *N*) were added. For shell valves and soft organs 4 parts of the acid were added to 1 part of the material. The extract was denatured with the equal amount of 20 per cent trichloroacetic acid solution.

It was found that the content of uric acid in *Mytilus edulis* varies much with the part of the body. Very interesting is that the foot is as rich in uric acid as the byssal tuft. Deducing from the content in the total soft body the parts other than foot may not be very rich in uric acid. Among the parts investigated the gill lamellae were the poorest in uric acid content.

Table 7.

Uric acid content, γ per 100 mg, of shell valves, soft parts, and byssal tuft of *Mytilus edulis*. For details of experimental procedure see the text. (Experiment 7)

Organ	Urib acid γ per 100 mg
Gill	5.9 4.0
Mid-gut gland	10.0 6.9
Foot	70.0
Total soft body, without byssus	7.4
byssal tuft	69.0
Shell valve	16.3 10.0 16.4

In this experiment larger specimens were used. To get 2 g of branchial tissues, however, 4 individuals were needed ; for 2.5 g of mid-gut gland 3 individuals and for 0.9 g pedal tissues 6 individuals were needed.

Experiment 8.

To confirm the localized distribuaion of uric acid in foot and byssus these parts were investigated (1 part of material was extracted with 99 parts of 3.5 *N* sulfuric acid). The soft body, of which foot and byssal tuft were removed, were also investigated. (1 part extracted with 19 parts of acid). There seems to be considerable fluctuation in the uric acid content of these parts. Moreover, there might be considerable errors due to the method of extraction, for in most cases the volume or concentration of the acid was not sufficient to dissolve all the tissues and their products within limited hours of heating. In spite of all these, however, quite evident is that the foot and the byssus are definitely richer in

Table 8.

Uric acid content, γ per 100 mg, of foot, byssal tuft, soft body without foot and byssal tuft, and shell valves of *Mytilus edulis*. For details of experimental procedure see the text. (Experiment 8)

Organ	Uric acid γ per 100 mg	Remarks
Foot	126.0	0.1 g from 3 small individuals
	80.0	0.2 g from 2 large individuals
Byssal tuft	116.0	0.2 g from 2 small individuals
	132.0	0.2 g from a large individual
Soft body without foot and byssal tuft	14.0	smaller specimen
	13.2	smaller specimen
	19.0	larger specimen
Shell valve	16.0	a single valve weighed 6.5 g
	17.2	〃 〃 5.6 g
	9.6	〃 〃 4.9 g
	10.4	〃 〃 5.8 g
	14.8	〃 〃 1.6 g
	14.8	〃 〃 1.4 g

uric acid than other parts (Table 8).

In this experiment two groups of specimens, larger and smaller, were used, but no relation between the uric acid content and the size or age of animal, as was suspected by comparing results of former two experinmets, was observed. The volume of sulfuric acid seems to be important to dissolve out completely the uric acid in the material. More exact investigations toward this point, however, will be carried out in future.

DISCUSSION AND CONCLUSION

From the results described in the first part of this paper it may be evident that the mussel, *Mytilus edulis*, and the oyster, *Ostrea gigas*, are quite short of urea-deaminating activity. Other bivalves, as the author has investigated in his previous works (Ishida 1954 b ; 1955 a, b), showed obvious activities in their tissue extracts to decompose urea under the conditions similar to those given to the present species, as investigated by the similar method. At least from the comparative point of view the mussel and the oyster may not be the animals which excrete ammonia in a copious amount. The author is not yet in the place to deny all the possibility of the occurrence of urease in these species, but he may say that there exist many other bivalved molluscs which are more adequate to be listed as examples of urea-decomposing species.

These two species, the mussel and the oyster, belong to different orders, but in respect of the activity of urea-deamination they are like each other. On the other hand, *Anadara inflata*, the member of Filibranchia to which *Mytilus edulis*

also belongs, showed a very high activity of urea-deamination as did eulamellibranchs such as *Mactra sulcataria*, *Meretrix meretrix lusoria*, and *Venerupis philippinarum*. No relation could be found between the occurrence of this activity and the taxonomic position of the animal.

One may postulate that the mussel and the oyster resemble each other in their ecologic characters, for both are sedentary and inhabit littoral, intertidal zones. In such a habitat the animals may sometimes be exposed to air and may have to endure a certain period of shortage of water. It may be advantageous under such circumstances to avoid production of free ammonia in the body which temporarily will be cut off from the surrounding aquatic world, and to keep catabolites in less soluble forms. However, no conclusive comment should be allowed without further investigations.

The author does not know anything about the fate of uric acid with precision, but, as cited above, *Mytilus edulis*, like *Ostrea gigas* and *Anadara inflata*, is less endowed with the activity to decompose uric acid, and this suggests to the author that most part of the purines in this animal may cease to be broken down by the stage of uric acid. On the other hand, an incidental observation suggested a possible increase of uric acid in the mid-gut gland of this species. If really this occurs in the living body, and if really the decomposition of uric acid does not take place in a noticeable degree, this substance must be rejected as such either in the form of urine or taking the form of *Speicherniere*.

The results of experiments described in the second part of this paper seem to show that *Mytilus edulis* has an evident tendency to accumulate uric acid in various parts of its body, especially in a very remarkable degree in the foot and byssus, in a less degree in shell valves, and in a lesser degree in other soft parts.

Whether does this substance exist in free form or as urate is not investigated yet. The high content of uric acid in the byssus may suggest a certain significant role played by this substance in making up that strong, elastic thread. The foot, which is in close relation in function with the secretion of byssal substances, may act as the reservoir of uric acid. Of course many detailed observations are needed before establishing any concrete ideas in this connection.

According to Kato and coworker uric acid and guanine are found as crystals in the connective tissues around the crystalline style sacs of certain gastropods (Kato and Kubomura 1955). They also suggest the occurrence of similar crystals in the similar tissue of lamellibranchs. Further they assume that uric acid crystals, which are formed and accumulated inside the connective tissue cells, will be discharged into intercellular spaces where they will be converted, by the action of unknown enzymes, presumably through xanthine, into guanine. Now, accordnig to these workers, the crystals of guanine are discharged into the crystalline style sac and then transported by the ciliary action into the alimentary tract.

According to these workers, therefore, guanine is assumed to be synthesized from uric acid in the molluscan tissues.

The present author does not know how could such a process be possible, but he is much interested in that the crystals of urate have actually been observed by them either in gastropod or lamellibranch tissues.

It is not seldom that animals accumulate uric acid somewhere within or outside their bodies. Either marine or land snail is known to excrete this substance. In lamellibranchs, however, there seems to have been no report except the one by Kato and coworker to give evidence to the excretion of uric acid out of the cells.

On the basis of the results of observations as described in this paper the present author assumes that certain kinds of bivalved molluscs are short of enzymes which catabolize purines to the last possible products, and instead, as he assumes, some of these animals may form, either starting from nucleic acid or from amino acids, purine compounds. These substances, as he further assumes, may be accumulated by certain kinds of bivalves in certain parts of their bodies, sometimes as important constituents in structures of high utility. *Mytilus edulis*, as the author assumes, may make up uric acid from certain source or sources, may use it as a constituent of byssus and shell valves, and may store it mainly in the tissues of foot which functions in coordination with the byssal secretion. Shell valves of this mollusc may also be assumed as an important depot of uric acid, for they contain this substance in a higher concentration than do the valves of other species.

Only a few experiments have been carried out as regards the uric acid content in the oyster. So far investigated, this animal does not seem to have any part in which uric acid is accumulated. The lack of the activity of urea-deamination, as well as the low activity of uricase, calls for further studies to elucidate the fate of uric acid in this shellfish.

Other kinds of bivalves should also be investigated in this regard.

SUMMARY

1. Deamination of urea by tissue extracts of various organs in the mussel, *Mytilus edulis*, and the oyster, *Ostrea gigas*, was investigated.

2. No positive result to prove the activity of urea-deamination could be obtained in either species.

3. Uric acid content of shell valves and other parts was determined in some bivalves.

4. The shell valves of *Mytilus edulis* showed the highest concentration of uric acid above those of others such as *Anadara inflata*, *Meretrix meretrix lusoria*, *Venerupis philippinarum*, *Mactra sulcataria*, and *Ostrea gigas*.

5. The soft body of *Mytilus edulis* contained more uric acid than that of *Ostrea gigas*.

6. Among various parts of *Mytilus edulis* the byssus and foot showed the highest and extraordinary content of uric acid.

7. Excretion in molluscs was discussed and the possible uricotelism in lamellibranchs was assumed.

LITERATURE

BRUNEL, A. 1938 C. R. Acad. des Sc. (Paris), **206** : 858. (Cited from Florkin 1945.)

FLORKIN, M. 1945 L'évolution du métabolisme des substances azotées chez les animaux. Actualités biochimiques, No. 3. Paris et Liège.

ISHIDA, J. 1943 Non-precipitable nitrogen in frog skin with special reference to purine bases. J Fac. Sci. Imper. Umiv. Tokyo. Sect. 4 (Zool.) **6** : 231 253.

ISHIDA, S. 1954 a Metabolic patterns in bivalves. I. Distribution of the activity to deaminate guanine in the soft parts of the clam, *Meretrix meretrix lusoria* (Gmelin). Sci. Rep. Tohoku Univ., 4th Ser. (Biol.) **20** : 249-255.

———— 1954 b Ditto II. Distribution of the activity to deaminate urea in the soft parts of the clam, *Meretrix meretrix lusoria* (Gmelin). *ibid.* 256-259.

———— 1954 c Ditto III. Supplenemtary and miscellaneous notes to the physiology of *Meretrix meretrix lusoria* (Gmelin). J. Coll. Arts and Sci. Chiba Univ. **1** : 4-43.

———— 1955 a Ditto IV. The urea-deaminating activity in *Corbicula*. Sci. Rep. Tôhoku Univ., 4th Ser. (Biol.) **20** : 327-331.

———— 1955 b Ditto VI. A preliminary survey into the distribution of the activity to deaminate urea in the soft parts of marine bivalves, *Anadara inflata Mactra sulcataria* and *Venerupis philippinarum*. Bull Mar. Biol. Sta. Asamushi **7** : 50-56.

———— 1955 c Ditto VII. Some aspects of the purine metabolism in the clam, *Meretrix meretrix lusoria* (Gmelin). J. Coll. Arts and Sci. Chiba Univ. **1** : 270 271.

———— 1956 Ditto VIII. Distribution of the guanine deaminating activity in the soft parts of some marine bivalves. Bull. Mar. Biol. Sta. Asamushi, **8** :9–18.

ISHIDA, S. and K. TSUZUKI 1955 Ditto V. Distribution of the uricase activity in the bivalves, *Meretrix meretrix lusoria* and *Venerupis philippinarum*. J. Coll. Arts and Sci. Chiba Univ. **1** : 267–269.

———— 1956 Ditto X. Distribution of the uricase activity in the bivalves, *Mactra sulcataria, Anadaria inflata, Mytilus edulis*, and certain other Species. J. Coll. Arts and Sci. Chiba Univ. **2** : 79–84.

KATO, K. and K. KUBOMURA 1955 Uric acid and guanine excretion from the crystalline style sac in some gastropods. Sci. Rep. Saitama Univ., Ser. B **2** : 21–234.

BULLETIN OF THE MARINE BIOLOGICAL STATION OF ASAMUSHI,
VOL. VIII, NO. 1. 1956

THE INFLUENCE OF TEMPERATURE ON THE SKIN POTENTIAL OF THE NEWT.*

By

Toyoo Aikawa
相 川 豊 夫
Marine Biological Station of Asamushi, Aomori Prefecture, Japan.

(With 2 Tables and 6 Figures)

Many investigations dealing with problems on the skin potential have been made. From the end of the ninteenth century, it has been proved that the ingoing potential might arise in the frog skin. Hashida (1922) and Uhlenbruck (1924) suggested respectively the absorption theory and the membrane theory on the electromotive phenomena of frog skin. Moreover, Hashida obtained important conclusions that the skin potential was intimately concerned with concentrations of contact electrolyte solutions. Amberson and Klein (1928) suggested that the membrane potential might be related to electrical properties of proteins. Effects of many ions on the skin potential were investigated by Francis (1934), Deans (1939) and others; meanwhile Williams and Sheard (1932), Francis and Pumphrey (1933) and Motokawa, Narita and Endo (1943) suggested that there were many important relations between the skin potential and the oxygen consumption. Francis and Pumphrey and others tried further investigations on the seasonal variation and the sexual differences of the skin potential.

According to Motokawa *et al.* (1943), the skin potential was affected by the temperature of the contact solutions; they derived a following experimental formula:

$$E = a + b\,T\,,$$

(E: electromotive force, a and b: negative and positive constants, T: absolute temperature). They obtained a rectilinear relation between E and logarithm of oxygen consumption at temperatures below 25°C.

Meyer and Bernfeld (1946) investigated the ionic permeability of frog skin by measuring the potential, and carried out an analysis of layers of the skin. The frog skin is composed of some layers with differect permeabilities, particularly the basal membrane has a special permeability for H ion, and the resting potential may arise with the difference of H ion concentrations on both sides of the

* Contritbutions from the Marine Biological Station of Asamushi, No. 222.

membrane.

In the work of previous authors the frog skin has exclusively been used. But the present work deals with the skin potential of the newt. The study was performed at the Biological Institute of the Tôhoku University in 1952.

I wish to express my sincere thanks to Prof. S. Nomura for his constant guidance and criticism in the course of this study.

MATERIAL AND METHOD

The newt, *Triturus pyrrhogaster* (Boie)*, collected in the environs of Sendai, was used, The dorsal side of the newt is black and the ventral side is red with black flecks. When two pieces of skin, about 2 cm^2, were cut off from the ventral, one was taken from the anterior black part, another from the posterior red part, and *vice versa*. Four combinations of parts and colours could be obtained.

The skin piece was mounted on an apparatus which is shown in Figure 1. The apparatus consisted of symmetrical glass chambers, 4 cm in length and 1.2 cm in diameter. In each chamber a capillary was inserted from the flank to bubble air into the solution. The surfaces, on which the skin was mounted pressed from both sides, were exactly plane parallel, and in the middle of the end surface, a

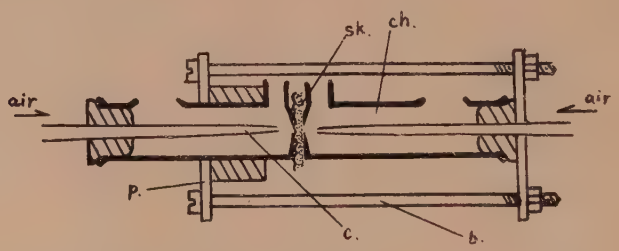


Fig. 1. Diagram of the apparatus. sk : skin, ch : glass chamber, c : capillary, b : screw-bolt, p : brass-plate.

small hole, 0.4 cm in diameter was made for the conduction through the skin. The hatched parts were of rubber, and the set was supported with two small brass plates, kept in position by three long screw-bolts. The chamabers of borh sides were always filled with Ringer solution of the same temperature and composition, through the hole 1 cm in diameter on the upper side. Figure 2 shows the conduction arrangement as follows : Ringer solution in chamber/Ringer agar-bridge/Ringer solution/sat. KCI agar-bridge/sat. KCI solution/sat. KCI calomel electrode ; this arrangement was also perfectly symmetrical. Then the potential was measured by the compensation method with the Type K potentiometer of

* Synonym : *Diemyctylus pyrrhogaster* (Boie)

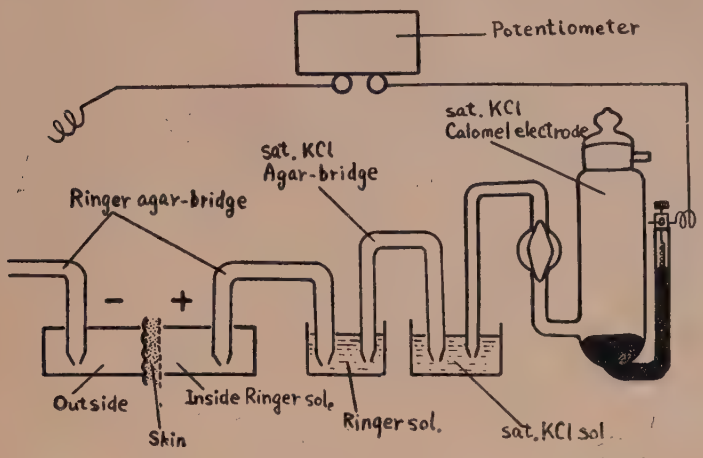


Fig. 2. The experimental arrangement for the determination of skin potential.

Leeds & Northrup, with a critical resistance galvanometer as a null instrument. The observational error would be $\pm$ 0.05 mV.

The Ringer solution on both sides of the skin could be rapidly removed and replaced by other solutions through the upper holes of the chambers with a syringe. The air bubbled from the capillaries went out through the upper tubular vent. The Ringer solution for frog, was modified with addition of $MgCI_2$: NaCI 0.65, KCI 0.014, $CaCI_2$ 0.012, NaH_2PO_4 0.003, $NaHCO_3$ 0.02, $MgCI_2$ 0.005, H_2O 100, buffered at pH 7.0–7.3.

RESULTS AND DISCUSSION

The newt skin potentials of 5–70mV, which were lower than that of the frog skin, were measured, the inner side being electrically positive as in the frog. The mechanism of rise of the skin potential has complicated relations with outer and inner conditions on the specimens, *i. e.*, temperature, light, form and concentration of contact electrolyte solution, tension of skin and vitality of newt itself. These conditions were kept constant throughout the experiment as far as possible.

In every observation, the potential changed for the first one to four hours after mounting, but with the progress of time a stable potential was finally attained. In the present study, the potential was measured at every ten minutes during five hours or longer. When the potential change was less than one mV per hour, the potential was taken as a steady potential.

To attain the steady potential, it might be necessary that concentrations of ions in Ringer solution of both sides had been defined, namely, the quantity of ions passing through the skin would attain an equilibrium (Donnan's membrane equilibrium).

To keep the equilibrium it was required that the skin must continue a constant metabolism (oxygen consumption, etc.), for which the experiments were facilitated by usual saturating with air constantly bubbled near the skin on both sides. If it was not done so, the potential was decreased and often to zero ; these phenomena might be connected to fatigue and death of skin. This was in agreement with the view that was concluded on the frog skin by Francis and Pumphrey (1933), Ponder and Macleod (1937) and Meyer and Bernfeld (1946).

The renewal of the Ringer solution during the first one to two hours increased the potential rapidly ; after the steady potential was attained, however, no effect of the renewal of solution could be observed.

(1) The Influence of Temperature on the Potential of the Skin Specimens cut off from Anterior and Posterior Parts of the Ventral Side of the Newt.

The experiment was carried out in October to January of the next year. The newts were cultured in thermostats controlled at 10°C and 25°C during 50 days, and the potentials of two skin pieces from each individual were measured in two sets of the apparatus at the same time. The experimental temperature was controlled at 10°C and 25°C as in culturing. Table I gives the results of measuring the potential, while Figures 3 and 4 show the curves of the potential changes in the time course. Figure 3 shows the curves of potential changes in 10°C animals, figure 4 those in 25°C animals, each measured at 10° and 25°C respectively.

a) 10°C-*Culturing and* 10°C-*Experiment.* As shown in Figure 3, curves of potential changes of the specimens cut off from the anterior and the posterior parts

Table 1.

Temperature influence on the skin potential of newt.

Temp. °C Cultur.- Exp.	No. of Exp.	Part & Colour of Skin: Anterior (Red) Initial transient Max. Steady P. mV	Part & Colour of Skin: Posterior (Black) Initial transient Max. Steady P. mV	No. of Exp.	Part & Colonr of Skin: Anterior (Black) Initial transient Max. Steady P. mV	Part & Colonr of Skin: Posterior (Red) Initiāl. transient Max. Steady P. mV
10–10	10	26.2 26.5 10.6	13.0 13.3 10.3	16	15.0 18.7 8.6	10.4 11.0 9.3
10–25	15	26.4 29.0 9.5	20.1 21.5 10.0	11	17.6 20.1 9.2	16.0 16.7 9.7
25–10	13	34.0 42.8 33.4	31.5 35.2 34.0	13	37.0 52.6 30.0	28.3 32.0 31.6
25–25	11	58.0 64.2 22.7	30.2 30.7 20.9	15	40.7 60.9 18.9	39.1 40.2 21.0

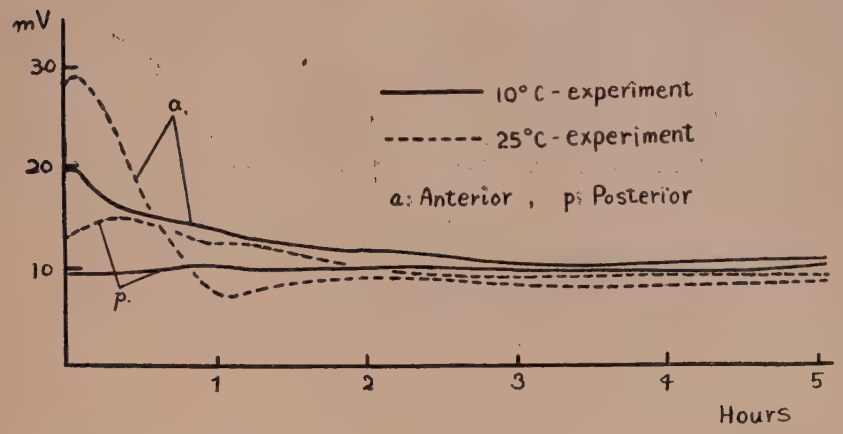


Fig. 3. Influence of temperature on the skin potential chahge in time course ; 10°C animal.

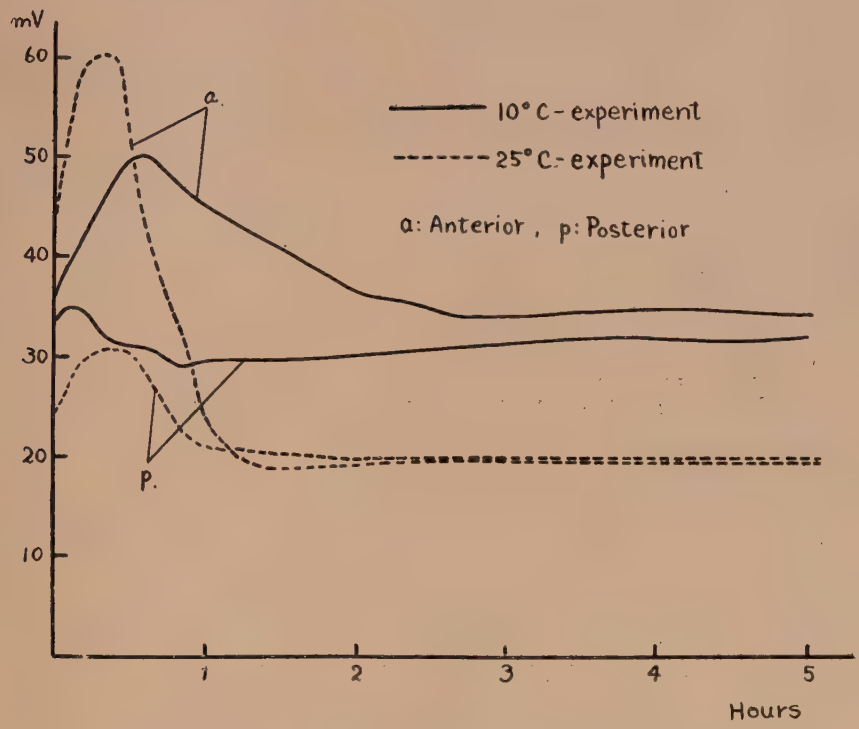


Fig. 4. Influence of temperature on the skin potential change in time course ; 25°C-animals.

were smooth, and showed slight changes for the first three to four hours; the steady potential of 5–20 mV was obtained. There was no difference between the black and the red pieces of skin, but the initial potential of the anterior might be slightly higher than that of the posterior.

b) *10°C-Culturing and 25°C Experiment.* The curves shown by broken line in figure 3, gives a clear tendency. In the first, the high potential of 20–30 mV was obtained for the anterior piece, but it fell suddenly after slight increasing. The potential of the posterior piece was about 13 mV at first, rose and fell gradually and reached the steady potential of 9 mV. The steady potential of anterior and posterior pieces was as low as in the 10°C-experiment, but the time for attaining the steady potential was only one to two hours. The difference between the black and the red pieces could not be found.

c) *25°C-Culturing and 10°C-Experiment.* The newts cultrued at 25°C were very active, compared with the newts at 10°C. In this case it was observed that the steady potentials were higher and the potential change was greater. The result is shown in curves of full line in figure 4. Either red or the black piece from the anterior, showed the larger potential change than those of the posterior; the result was always decisive. It required three to four hours to attain the potential, which is 30mV or higher.

d) *25°C-Culturing and 25°C-Experiment.* The result was shown with broken line in figure 4. In this case, the steady potential was attained in first one to two hours, differing from 10° C-experiment, being always comparatively low, 10–25 mV. The results obtained in the foregoing experiments show no difference between the black and the red but might present characteristic difference between the anterior and the posterior. In 25° C-culturing and 25° C-experiment, the largest changes and the highest values of initial potentials were obtained, followed by the rapid decreases.

Though the maximum potentaial of frog skin was obtained in experiments at 25°C by Barnes and Motokawa, it might be nothing but the structure of newt skin that made difference from the frog. There might be a remarkable agreement between the results and the observations in which Motokawa obtained the low potential on the frog at the experimental temperature of 30°C.

Though the skin potential must be thought as steady, the processes of the potential changes are noticeable in the present observations. It seems likely that curves of the processes suggest a few characters of skin potentials and if the curves are considered in relation to difference of constitution fetween the anterior and the posterior parts, then each character might be discovered in the curves. Moreover, when the curves obtained under temperature control are compared with curves of annual potential variation after stated, resemblance might be found between them.

When a steady potential was attained in 25°C-culturing and 25°C-experiment, temperature of Ringer solution was changed. The medium on both sides of the skin set in the apparatus was replaced by Ringer solution of 10°C, and a rapid decrease of about 15 mV was observed in the anterior skin and 10 mV in the posterior. The decrease was followed by the steady potential immediately in the anterior skin, and after an increase of two to three mV in the posterior. After two hours, the Ringer solution was removed and replaced again by solution at 25°C on both sides, and it was observed that the steady potential was obtained after immediate increase of about 10 mV followed by slight decrease for one hour in the anterior skin. In the posterior skin the steady potential was attained after increasing of about 10 mV followed by decreasing of two to three mV in the following five minutes. The result is in agreement with the point that has been obtained on the frog skin by Barnes (1939), though the degree of increase and decrease in the newt skin may be smaller and types of change may differ from those of the frog. It was concluded that the skin potential of newt was intimately influenced by the temperatures of culturing and experiments, but the decidedness and the degree of influences of temperature would be less than in the frog. In the newt it seems that the maximum potential is obtained about 15–20°C, and that the steady potential will be reached sooner at higher experimental temperature. These results are in agreement with Motokawa's observations.

(2). The Skin Potential of the Newt and the Season.

It was likely that seasonal variation might exist in the skin potential of the newt measured from July to the second third of January of the next year. This experiment was made at the room temperature with the ventral skin of the newt freshly collected in every month. Table II gives the average, maximum and minimum values in every month. Figure 5 shows typical curves which were most frequently obtained in each month. From Table II and figure 5, it may be stated

Table 2.

Seasonal variation of steady potential of the newt skin.

Month	Steady Potential mV		Month	Steady Potential mV	
	Average.	Max. & Min.		Average.	Max. & Min.
July	**32.8**	69.3 24.7	November	**9.5**	20.8 2.7
August	**27.1**	51.4 18.6	December	**7.7**	16.4 2.5
September	**19.7**	32.5 9.8	January I.	**8.0**	24.3 3.2
October	**13.9**	20.0 3.5	January II.	**16.5**	30.9 8.8

that the steady potentials were decreasing from summer to winter ; furthermore, compared with the results, which were obtained from the foregoing culturing and experiments under temperature control, many analogies were found in types of

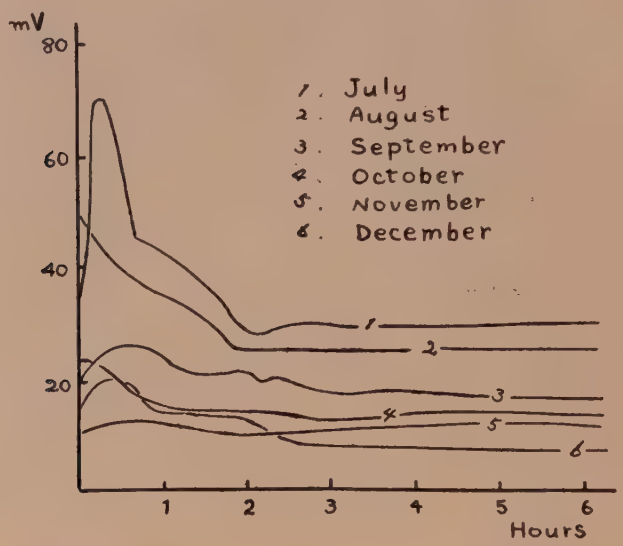


Fig. 5. Seasonal variation of the skin potential.

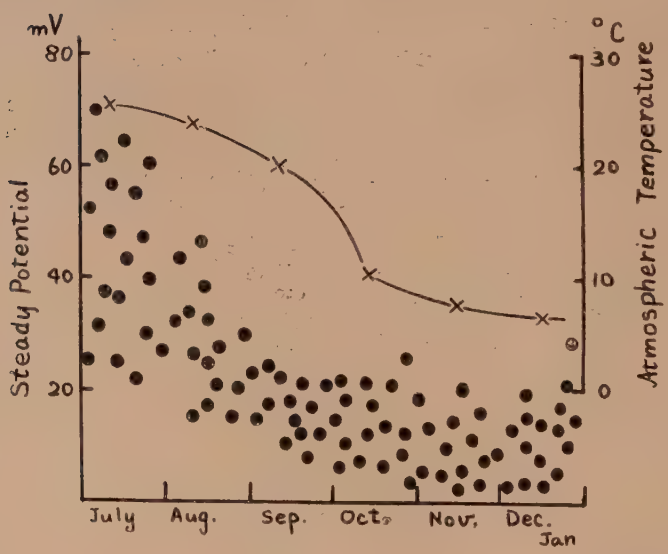


Fig. 6. Seasonal variation of the steady potential of the newt skin. The above curve shows atmospheric temperature in each month, and every black circle gives average value of the skin potentials of 2-3 individuals.

curves and values of potentials measured in the seasnoal experiment. It seemed likely, therefore, that these seasonal variations certainly were related mainly with the temperature. The relations between the steady potential and the atmospheric temperature in every month, are given in Figure 6. As shown in this figure, the range from maximum to minimum was about 70 to 25 mV in July, but in August the maximum descended to 45 mV. In September the mimimum fell to 10 mV and also the maximum was not so high, about 30 mV ; while at this time the atmospheric temperature was falling gradually to about 17°C ; the range between maximum and minimum potentials in this season became narrower. From October on, the atmospheric temperature fell down suddenly, and it was observed that the lower potentials such as 5–6 mV were often measured. Potentials in November were as low as in October. In December and the first ten days of January, the minimum potential throughout the year might be observed ; in the middle of January, a slight increase of the potential was observed and the maximum in the days attained to 20 mV, rarely 30 mV high.

When these results are considered in connection with the observations in the experiments on the temperature, and moreover, compared with the investigation by Francis and Pumphrey (1933) in which it was reported that the maximum potential of the frog skin is observed in February to May and the sexual difference appears from January to March, it will be noticed that there are remarkable agreements. The relation between the seasonal potential variation and the potential change by controlling the experimental temperature is perhaps the most interesting results of the present studies.

Though the difference between male and female has not been observed throughout the present study whcih extended from July to January, yet the sexual difference in the characteristics of the black and the red parts of the skin might have been observed, if the potential had been measured in January to May, because this term is the breeding season of the newt, and the greatest sexual difference in skin potential of the year might have been observed.

As on the frog skin potential, further experiments on the newt skin related with physiological and histological studies throughout the whole year should be carried out for elucidation of the problems.

SUMMARY

1. The skin potential in the newt was measured by the compensation method with Ringer solution as conduction medium on both sides of the skin set in the specially devised apparatus.

2. Skin pieces were prepared from different parts of anterior and posterior regions of the ventral side of the trunk of the newt, each being either red or black in colour ; four combinations of regions and colours could be obtained.

3. The potential was positive on the inside, and its value was about 5 to 70 mV.

4. The initial potentials at first changed rapidly, but gradually tended to steady potentials.

5. As far as the steady potentials are concerned, there has been observed no remarkable difference between red and black parts of the skin, but the time course of the potential changes showed characteristic difference between the anterior and the posterior parts of the skin.

6. The influences of the temperature in culturing and experiment might be observed on every curves, and the time required to attain the steady potential was shorter at higher experimental temperatures.

7. By the replacement of Ringer solution of different temperature (25° to 10°C and 10° to 25°C) on both sides of the skin, the steady potential sensitively changed. Increase and decrease of about 10–15 mV were observed.

8. It is noticeable that seasonal variations take place in the skin potential of the newt ; when measurements were carried out in July to January of the next year, decrease of the steady potential was observed, which might intimately be influenced by the atmoshperic temperature.

REFERENCES

Amberson, W. R. and H. Klein 1928 The influence of pH upon the concentration potentials across the skin of the frog. Jour. Gen. Physiol., **11** (6) : 823-841.

Barnes, T. C. 1939 The influence of heavy water and temperature on the electrical potential of frog skin. Jour. Cell. Comp. Physiol., **13** (1) : 39-50.

Deans, R. B. 1939 Potentials and effective ionic mobilities in frog skin. Jour. Exp. Biol., **16** (2) : 134-142.

Francis, W. L. and R. J. Pumphrey 1933 The electrical properties of frog skin. Part I. Jour. Exp. Biol., **10** (4) : 379-385.

Francis, W. L. 1934 The elecrrical properties of isolated frog skin. Part II. Jour. Exp. Biol., **11** (1) : 35-47.

Hashida, K. 1922 Untersuchungen über das elektromotorische Verhalten der Froschhaut. I. Die Abhängigkeit des elektromotorischen Verhaltens der Froschhaut ableitenden Flüssigkeiten. Jour. Biochem., **1** (1) : 21-67.

Meyer, K. H. and P. Bernfeld 1946 The potentiometric analysis of membrane structure and its application to living animal membranes. Jour. Gen. Physiol., **29** : 353-378.

Motokawa, K., T. Narita and N. Endo 1943 Das bioelektrische Potential und der Sauerstoffverbrauch der Froschhaut in Abhängigkeit von der Temperatur. Jap. Jour. Med. Sci. III, Biophysics, **8** (4) : 181-193.

Ponder, E. and J. Macleod 1937 The potential and respiration of frog skin. Jour. Gen. Physiol., **20** (3) : 433-447.

Steinbach, H. B. 1933 The electrical potential difference across living frog skin. Jour. Cell. Comp. Physiol., **3** (1) : 1-27.

Williams, M. M. and C. Sheard 1932 The effect of x-rays on the electric potentials and the rate of oxidation of frog's skin. Protoplasma, **15** : 396-414.

BULLETIN OF THE MARINE BIOLOGICAL STATION OF ASAMUSHI,
VOL. VIII, NO. 1. 1956

OCEANOGRAPHICAL OBSERVATIONS OFF ASAMUSHI DURING 1954*

By

BUNRYÛ TSUBATA
津 幡 文 隆
Marine Biological Station of Asamushi, Aomori Prefecture, Japan

(With 4 Figures and 1 Table)

In the present article, the results of oceanographical observations carried out at a definite station off Asamushi as the routine work during 1954 were described.

The observations were made in the same way as that employed in the previous works, and 22 series of observations were made during this year.

Acknowledgements are due to Prof. Dr. S. Nomura, Director of the Marine Biological Station of Asamushi, Tôhoku University, for his constant guidance throughout the course of the works.

RESULTS

1) *Water Temperature* (Fig. 1, Table 1)

In 1954, the minimum temperature at the surface appeared early in March, and at 5, 10, 20 and 30 metre depths in the middle of March. The value of the minimum temperature below 5°C was lower than that in the normal year. Referring to the past data, it was found to be the fourth observation since 1927, as shown in the following table, that the cold water mass below 5°C was measured in the period of the minimum temperature.

			Depth				
No.	Year	Date	0	5	10	20	30
1	1927	Mar. 5	4.3	4.2	3.9	**3.8**	**3.8**
		Mar. 13	**3.3**	3.4	3.4	3.5	3.5
		Mar. 30	3.9	3.9	3.8	3.8	**3.7**
2	1931	Feb. 28	**4.8**	5.0	4.9	4.9	5.0
3	1948	Feb. 12	**4.60**	4.80	4.80	4.90	4.80
4	1954	Mar. 1	**4.71**	5.30	5.31	5.32	5.34
		Mar. 18	5.01	4.91	**4.66**	4.71	4.96

* Contributions from the Marine Biological Station of Asamushi, No. 223.

Table 1

Oceanographical Conditions Observed at Definite

No. of Observations	Date	Water Temperature (°C)					Chlorinity (‰)					Oxygen (cc/L)		
		0	5	10	20	30	0	5	10	20	30	0	5	10
1	Jan. 14	8.83	8.83	9.80	9.50	9.32	18.54	18.54	18.58	18.63	18.64	6.34	6.37	6.33
2	Feb. 2	6.25	6.41	6.61	6.62	6.65	18.54	18.54	18.62	18.62	18.62	6.73	6.79	6.71
3	17	5.40	5.51	5.72	5.71	5.30	18.25	18.47	18.57	18.57	18.58	7.09	7.10	7.18
4	Mar. 1	4.71	5.30	5.31	5.32	5.34	15.92	18.39	18.49	18.52	18.55	7.38	7.17	7.13
5	18	5.01	4.71	4.66	4.71	4.96	18.11	18.47	18.47	18.47	18.53	7.31	7.29	7.28
6	Apr. 2	5.97	5.49	5.41	5.11	5.45	16.75	18.37	18.40	18.47	18.53	7.19	7.18	7.10
7	17	7.33	6.66	6.29	6.29	6.41	17.03	18.13	18.27	18.32	18.49	7.11	7.11	7.11
8	May. 1	7.91	7.81	7.50	7.30	7.41	16.50	18.16	18.24	18.48	18.51	/	/	/
9	17	10.66	10.10	9.38	9.00	8.90	17.93	18.33	18.47	18.47	18.48	6.71	6.61	6.68
10	June 1	13.22	12.31	11.83	10.95	10.21	18.18	18.22	18.31	18.53	18.53	6.32	6.42	6.44
11	16	13.03	11.96	11.73	11.54	11.39	17.98	18.36	18.40	18.48	18.49	6.16	6.26	6.24
12	July. 15	17.31	16.43	15.55	13.64	13.54	17.88	18.23	18.36	18.51	18.65	5.62	5.68	5.88
13	Aug. 2	21.32	17.63	17.80	17.47	16.79	17.68	18.43	18.62	18.68	18.65	5.61	5.28	5.20
14	15	23.98	21.59	20.28	19.18	18.37	18.11	18.49	18.65	18.67	18.73	5.20	5.03	5.00
15	Sept. 1	21.80	21.78	21.44	20.43	19.80	18.45	18.46	18.45	18.74	18.80	5.32	5.30	5.27
16	15	23.70	22.67	22.09	21.50	21.16	18.14	18.55	18.60	18.63	18.63	5.13	5.17	5.11
17	Oct. 1	20.47	25.43	20.42	20.36	19.27	18.64	18.63	18.65	18.72	18.73	5.14	5.11	4.98
18	15	17.59	17.87	17.89	17.90	17.91	18.49	18.60	18.63	18.63	18.66	5.26	5.30	5.30
19	Nov. 9	14.81	15.42	15.41	15.41	15.41	18.49	18.65	18.67	18.68	18.68	5.62	5.69	5.59
20	20	13.53	13.55	13.55	13.55	13.60	18.63	18.63	18.63	18.63	18.63	5.70	5.69	5.56
21	Dec. 1	12.94	12.94	12.94	12.94	12.69	18.63	18.63	18.63	18.63	18.64	5.70	5.71	5.70
22	20	9.89	9.88	9.85	10.31	10.39	18.51	18.51	18.53	18.63	18.66	6.20	6.11	6.30

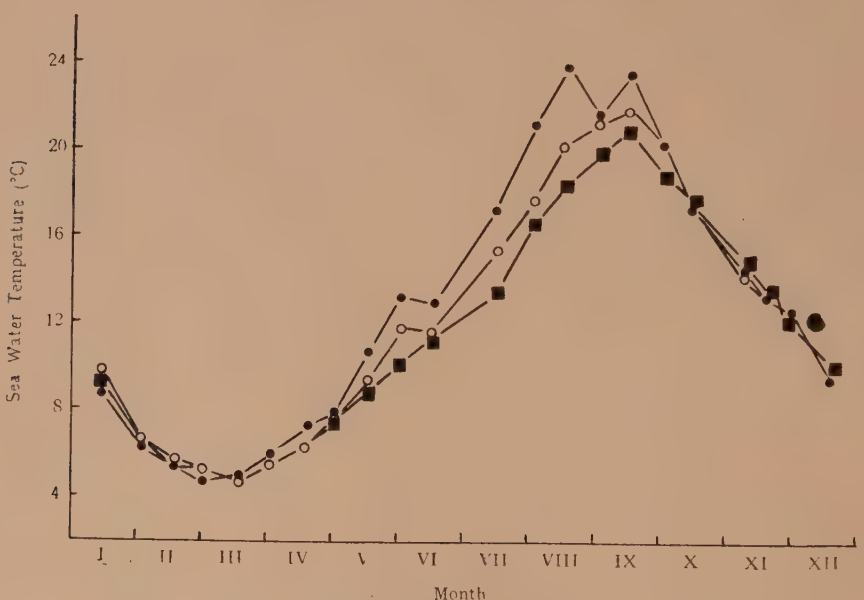


Fig. 1. Annual change of sea water temperature at different depths off Asamushi during 1954.
● .... 0 m layer, ○ .... 10 m layer, ■ .... 30 m layer.

Station off Asamushi During 1954.

Saturation of O_2, %							pH					Colour of Water	Transparency	Air Temperature
20	30	0	5	10	20	30	0	5	10	20	30			
6.29	6.30	95.7	96.2	97.5	96.2	96.1	/	/	/	/	/	3	11	−0.5
6.69	6.71	96.0	97.0	96.5	96.3	96.5	8.3	8.35	8.35	8.35	8.35	4	11	−1.5
7.10	7.09	98.6	99.6	101.4	100.1	99.2	8.35	8.35	8.35	8.35	8.35	4	12	2.0
7.07	6.55	98.1	100.1	99.6	98.7	91.6	8.35	8.35	8.35	8.35	8.35	5	3.5	4.5
7.24	7.22	100.8	100.3	100.1	99.6	100.1	8.35	8.35	8.3	8.3	8.3	5	10.5	7.5
7.06	6.82	99.6	100.4	99.4	98.1	95.5	8.35	8.35	8.3	8.3	8.3	5	10	8.0
6.99	6.89	102.0	102.0	101.0	99.0	98.9	8.3	8.3	8.3	8.3	8.3	5	10	12.3
/	/	/	/	/	/	/	8.3	8.2	8.2	8.2	8.2	5	11	14.0
6.61	6.54	104.0	102.0	101.0	100.0	98.9	8.2	8.2	8.15	8.2	8.1	5	10	14.3
6.57	6.33	103.0	103.0	103.0	102.6	98.7	8.3	8.2	8.2	8.2	8.2	3	10	16.9
6.19	6.25	100.4	100.0	100.0	98.8	98.8	8.3	8.3	8.3	8.3	8.3	5	8.5	13.5
5.86	5.82	98.8	99.3	99.8	96.9	96.8	8.2	8.2	8.2	8.2	8.3	4	10	18.6
5.30	5.20	105.0	93.8	94.3	94.0	91.4	8.3	8.3	8.2	8.2	8.2	4	7	21.8
4.86	4.70	102.4	96.0	93.5	89.0	84.8	8.3	8.25	8.2	8.2	8.2	3	8	28.2
5.39	4.66	101.4	100.8	100.0	100.2	86.2	8.3	8.3	8.3	8.3	8.3	5	12	25.0
5.08	5.02	100.6	100.0	98.0	96.7	94.8	8.3	8.3	8.3	8.3	8.3	3	7	28.2
4.92	4.77	96.1	95.1	93.0	92.0	87.6	/	/	/	/	/	4	7	20.2
5.30	5.28	93.4	94.8	95.0	95.0	94.6	8.3	8.3	8.3	8.3	8.3	4	11.5	14.6
5.53	5.52	95.1	97.3	95.5	94.9	94.7	8.3	8.3	8.3	8.3	8.3	4	9	13.3
5.50	5.61	94.2	94.0	92.0	90.8	93.0	8.3	8.3	8.3	8.3	8.3	4	13	12.6
5.60	5.49	93.2	93.7	93.2	91.6	89.4	8.4	8.35	8.35	8.35	8.35	3	17	11.8
6.15	6.03	95.8	94.5	97.0	95.8	94.0	8.3	8.3	8.3	8.3	8.2	4	11	8.2

Thereafter, water temperature in each layer rosed gradually. The maximum water temperature at the surface appeared in the middle of August and in the middle of September. For the water temperature of the surface early in September dropped down by the rain on August 28 and 29. The maximum value below 5 metre depth appeared in the middle of September.

As regards the vertical distribution of water temperature, the periods of January to March and October to December seem to be the period of inverse stratification.

2) Chlorinity (Fig. 2 and Table 1)

Observations on the variation of chlorinity in 1954 show that the variations at 10, 20 and 30 metre depths were inconspicuous, fluctuating between 18.24 and 18.67 ‰ at 10 metre depth, between 18.32 and 18.74 ‰ at 20 metre depth, and between 18.49 and 18.80 ‰ at 30 metre depth.

But the value of chlorinity at the surface layer was generally lower than that at the other layers and more variable throughtout the year, fluctuating between 15.92 and 18.64 ‰. The lowest value 15.92 ‰ was observed on March 1. The lowest value in the normal year has been observed in April or May. The value of chlorinity was low in April and May of this year too. However, it belongs to

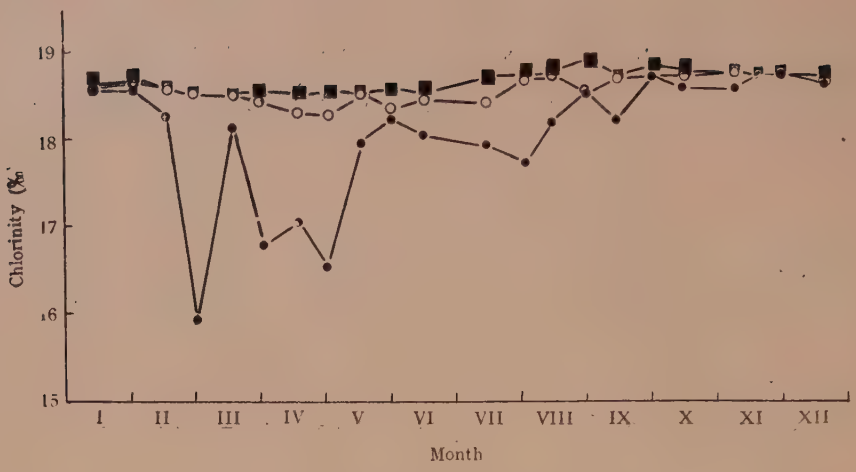


Fig. 2. Annual change of chlorinity at different depths off Asamushi during 1954.
● .... 0 m layer, ○ .... 10 m laryer, ■ .... 30 m layer.

a rare case that the lowest value was observed early in March.

The decrease of chlorinity appeared from June to August of this year, which seems to be caused by the rain.

During the period of inverse thermal stratification, the chlorinity of the surface showed almost the same value with that of the other layers.

3) Dissolved Oxygen (Fig. 3, 4 and Table 1)

As regards the seasonal change of dissolved oxygen in the normal year, the maximum value in every layer appears in the spring season, and the minimum

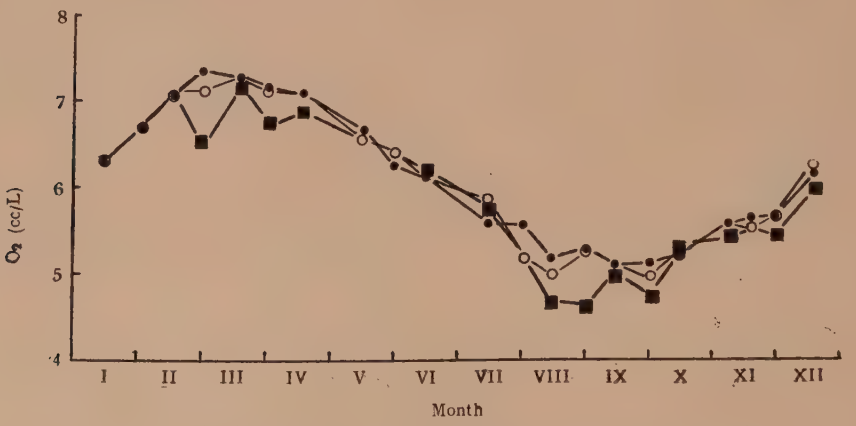


Fig. 3. Andual change of the O_2 content of sea water at different depths off Asamushi during 1954.
● .... 0 m layer, ○ .... 10 m layer, ■ .... 30 m layer.

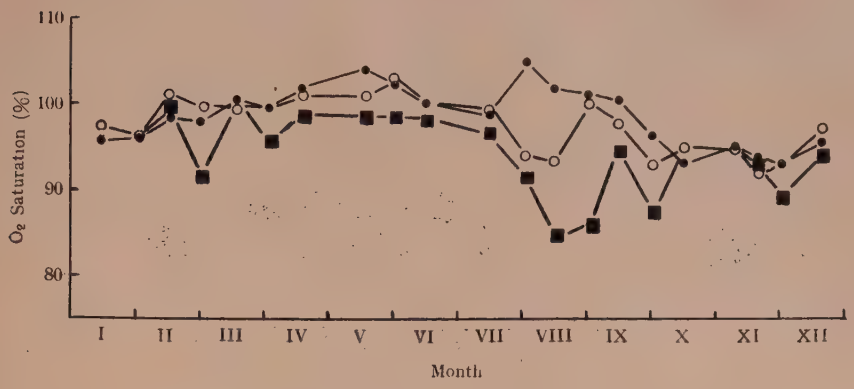


Fig. 4. Annual change of saturation of O_2 at different depths during 1954.
● ....0 m layer, ○ .... 10 m layer, ■ .... 30 m layer.

in the autumn season. This year did not differ much from the normal year, and the highest value of dissolved oxygen at the surface layer appeared on March 1, amounting to 7.38 cc, and the lowest value at 30 metre depth on September 1, amounting to 4.66 cc.

Considering the seasonal change of saturation of oxygen, oversaturation of oxygen in this year appeared generally between 0 and 10 metre depths from March to September, with some exceptions. Consequently, the percentage saturation of oxygen, seems to be lower in the period of inverse stratification and higher in the period of direct stratificatiohn.

SUMMARY

1. In the present article, the results of oceanographical observations at a definite station off Asamushi during 1954 were reported.

2. The minimum water temperature below 5°C was observed early in March. The value of water temperature was lower than that in the normal year. It belongs to a rare case at the definite station off Asamushi.

3. The minimum value of chlorinity was observed early in March which seems to be caused by the snow melt. It seems to be earlier than in the normal year.

LITERATURE

Kokubo, S. and T. Tamura 1938 Observations on the plankton and hydrographic conditions of Aomori Bay during the period 1933-1935. Records of Ocean. Works of Japan. **10** (1) : 1-15.

Okitsu, O. T. Tokui and B. Tsubata 1954 Oceanographic Obseevations off Asamushi during 1952. Bull. Mar. Biol. Stat. Asamushi, **7** (1) : 21-25.

TSUBATA, B. 1953 Report on the temperature and chlorinity of the waters off Asamushi during 1951. *ibid.* **6** (1-4) : 1-5.

———— 1955 Oceanographic observations off Asamushi during 1953. *ibid.* **7** (2, 3, & 4) : 152-158.

東北大学

淺虫臨海實驗所報告

（海洋生物時報 改題）

第七卷

昭和二十九・三十年

THE BULLETIN OF THE MARINE BIOLOGICAL STATION OF ASAMUSHI TÔHOKU UNIVERSITY

Vol. VII,

(1954, 1955)

Asamushi, Aomori Prefecture

JAPAN

東北大学附属臨海實驗所

青森縣淺虫

CONTENTS

No. 1 (Published March 1954)

Page

Eturô HIRAI
On the Row of Stigmata of the Ascidian, *Perophora orientalis* Ärnbäck 1

Eturô HIRAI
Morphological observations of Ascidian Larvae. I. *Cyathocormus* OKA, 1913 7

Tetsusaburô OKISTU
On the Plankton of Koikuchi-no-Ike 越口の池, Tsugaru Jûniko 津軽十二湖 11

Tetsusaburô OKISTU
On the Seasonal Change of *Ceratium* in Aomori Bay 17

Tetsusaburô OKISTU, T. TOKUI and B. TSUBATA
Oceanographical Observations off Asamushi during 1952 21

Nos. 2, 3, & 4 (Published March 1955)

Shichiroku NOMURA
The Thirtieth Anniversary of the Marine Biological Station of Asamushi, Tôhoku University 27

Shun-ichi TAKATSUKI
Contribution to the Physiology of the Heart of Oyster. VI. The Action of Nicotine on the Isolated Heart of Oyster 39

Tametake NAGANO
The Behavior of Crustacea Exposed to Colored Light 44

Shûzô ISHIDA
Metabolic Patterns in Bivalves. VI. A Preliminary Survey into the Distribution of the Activity to Deaminate Urea in the Soft Parts of Marine Bivalves, *Anadara Inflata*, *Mactra Sulctaria* and *Venerupis Philippinarum* 50

Mitsuo SATÔ
Studies on the Pit Organs of Fishes. III. The Distribution of the Large Pit Organs 57

Gotarô YAYAMOTO
On Rearing of the Scallop Spats in Tank and Pool 69

Satarô KOBAYASHI, Yoshio WAKU and Kenichi SUMIMOTO
Refractometric Studies on the Haemolymph in the Fasting

Silkworm, *Bombyx Mori* L. 74

Kunio HIRATA

The Existing and Raised Coral Reefs in Yoron Island and Their Shell Bearing Molluscs 89

Ikusô HAMAI

Geographical Distribution of Animals in the Japanese Islands with Special Reference to an Area-Factor and to the Closeness Among the Islands 100

Jirô TANAKA

Anatomical Observations on *Anomia Cytaeum* Gray 111

Takeo ITÔ

Studies on the Reproduction of Hydras. VIII. Causative Effect of Lowering Temperature on the Sexual Maturity in *Hydra Parva* Itô 120

Noboru ABE

Colony Formation of a Limpet, *Acmaea Dorsuosa* Gould, and Variation of Level of the Colony 127

Katsuhiro OKADA, Takeshi HASHIMOTO and Yasuyo MIYAUCHI

A Preliminary Report on the Poisonous Effect of the *Toxopneustes* Toxin Upon the Heart of Oyster 133

Wataru TESHIROGI

The Effect of Lithium Chloride on Head-Frequehcy in *Dugesia Gonocephala* (Dugès) 141

Singo NAKAZAWA

Staining Embryos of *Sargassum Confusum* Ag., A Brown Alga .. 147

Bunryû TSUBATA

Oceanographical Observations off Asamushi During 1953..... 152

Gunji TOMITA

On the Nomura and Tomita Method for Measuring the Mechanical Activity of Cilia 159

冨田軍二

纖毛の機械的活動性の"野村・冨田式"測定法について 164

東北大学附属臨海実験所創立卅周年記念祝賀記事 168

追 想 録

浅虫臨海実験所の思い出 ..... 畑 井 新喜司 177

東北大学浅虫臨海実験所 30 年の想出 ..... 小久保 清 治 178

浅虫臨海実験所の思ひ出 ..... 高 槻 俊 一 182

悪 童 の 手 記 ..... 永 野 為 武 183

臨海実験所の誕生 ..... 野 村 七 録 185

昭和31年3月26日 印刷
昭和31年3月31日 発行

編集兼
発行者　東北大学附属臨海実験所
青森県浅虫
電話 浅虫 45

印刷所　笹気出版印刷株式会社
仙台市堤通27番地
電話 ③ 2027, ③ 2207

CONTENTS

HIRAI, E. and B. TSUBATA. On the spawning of an ascidian, *Halocynthia roretzi* (DRASCHE) 1

HIRAI, E. Morphological observations of asidian larvae 2. *Chelyosoma siboja* OKA .. 5

HIRAI, E. Morphological observations of ascidian larvae 3. *Corella japonica* HERDMAN, VAR. *asamusi* OKA 7

ISHIDA S. Metabolic patterns in bivalves VIII. Distribution of the guanine-deaminating Activity in the soft parts of some marine bivalves 9

ISHIDA, S. Metabolic patterns in bivalves IX. Some aspects of nitrogen metabolism in marine bivalves, with special reference to the mussel, *Mytilus edulis* 19

AIKAWA, T. The influence of temperature on the skin potential of the newt 33

TSUBATA, B. Oceanographical observations off Asamushi during 1954 43

All communications intended for "THE BULLETIN OF THE MARINE BIOLOGICAL STATION OF ASAMUSHI" should be addressed to Prof. S. Nomura, the Biological Institute, Tôhoku University, Sendai, Japan.